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TITLE: Magnetic Resonance Spectroscopy Imaging and Functional
Magnetic Resonance Imaging of Neurofibromatosis Type 1:
In Vivo Pathophysiology, Brain-Behavior Relationships,
and Reading Disabilities

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13. ABSTRACT (Maximum 200 Words)

The purpose of this research is oriented towards understanding the reading, language, and articulation deficits associated with Neurofibromatosis Type 1 (NF-1) and relating these deficits to the underlying pathophysiology of NF-1 as revealed by Magnetic Resonance Spectroscopy Imaging (MRSI). A second goal is to determine how differences in activation, as measured by functional Magnetic Resonance Imaging (fMRI), are linked to the cognitive/academic impairments associated with NF-1. A third goal is to further understand how T-2 weighted hyperintensities on Magnetic Resonance Imaging (MRI) scans are related to cognitive/academic impairments associated with NF-1. Each aim addresses the research in terms of pathophysiology and how cognitive/academic functioning of children with NF-1 compares to control groups when examined in both genetic (i.e., sibling) as well as general population (both reading disabled and non-reading disabled) contexts. We hypothesize that abnormalities of NAA, Choline, or their ratios, will exist in the thalamus and will correlate with language, reading, and articulation deficits in NF-1, defined by "lowering" of the cognitive scores of each child with NF-1 relative to his/her unaffected sibling. For the second goal, we hypothesize that children with NF-1 will activate their brains similarly to reading disabled children during fMRI tasks. For the third goal, we hypothesize that reading, language, and articulation deficits will correlate with the number of brain locations with T2-weighted hyperintensities. Thus, neuroimaging permits the pursuit of furthering our understanding of how the NF-1 gene affects the brain in terms of basic neurobiologic factors (ultrastructural, physiological, and localization) as well as their impacts on cognition (reading, language, and articulation) in NF-1.

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Learning Disability Diagnosis, Neurofibromatosis Type 1, functional Magnetic Resonance Imaging, anatomical Magnetic Resonance Imaging, Magnetic Resonance Spectroscopy Imaging, Reading and Speech/Language disorder

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INTRODUCTION

The purpose of this research is primarily oriented towards understanding and documenting the reading, language, and articulation deficits associated with Neurofibromatosis Type 1 (NF-1) and relating these deficits to the underlying pathophysiology of NF-1 as revealed by Magnetic Resonance Spectroscopy Imaging(MRSI). A second goal is to determine how differences in activation, as measured by functional Magnetic Resonance Imaging (fMRI), are linked to the cognitive and academic impairments associated with NF-1. A third goal is to further understand how the brain's visible abnormalities, T-2 weighted hyperintensities on Magnetic Resonance Imaging (MRI) scans, are related to the reading, language, and articulation deficits in NF-1. Each of the specific aims of the research addresses components of the research in terms of pathophysiology and how cognitive/academic functioning of children with NF-1 compares to control groups when examined in both genetic (i.e., sibling) as well as general population (both reading disabled and non-reading disabled) contexts. Based upon previous research findings, we hypothesize that abnormalities of NAA, Choline, or their ratios, will exist in the thalamus; further, that thalamic abnormalities will correlate with language, reading, and articulation deficits in NF-1, as defined by the "lowering" of the cognitive score of each child with NF-1 relative to that of his/her unaffected sibling. In terms of the second goal of this research, we hypothesize that children with NF-1 will activate their brains similarly to reading disabled children during fMRI tasks. In terms of the third goal of this research, we hypothesize that reading, language, and articulation deficits will (as reported for IQ) correlate with the number of brain locations in which T2-weighted hyperintensities are seen. Thus, the use of MRI, MRSI, and fMRI methodology in this research permits the pursuit of further understanding the basic neurobiologic factors (ultrastructural, physiological, and localization) as well as their impacts on cognition (reading, language, and articulation) in NF-1, thus furthering our understanding of how the NF-1 gene affects the brain.

BODY

Research Accomplishments Associated With Each Task: Tasks 1 and 2, which were targeted for years one and two of the grant, have continued to be addressed during the second year of the grant. Task 1 dealt with subject recruitment and data collection (targeted for months 1-26), and included the goals of recruiting patients for participation, screening patients for eligibility, and conducting onsite neuropsychological evaluations and MRSI/fMRI procedures. We have seen a total of 49 patients (31 this year; see chart below), all of whom received neuropsychological testing; of these children, four children with NF-1 (sibling pairs) have participated in MRSI and two children with NF-1, three children with RD, and 1 control have participated in the fMRI tasks. Task 2 dealt with analyzing MRI data and scoring neuropsychological tests (months 3-26). We have analyzed MRSI data, fMRI data that we have collected and scored neuropsychological tests (including inter-rater reliability) that we have administered.

Tasks 3 and 4 (data entry, statistical analysis, and results/manuscript preparation), which were targeted for the end of year 2 and all of year 3, are currently being addressed as well. We continue to enter MRI and neuropsychological data into the database as we collect MRI and neuropsychological data. In addition, we have an accepted abstract on the neuropsychological findings that we will be presenting at the *International Neuropsychological Meeting* in February in Honolulu, Hawaii. We are also currently preparing a manuscript for publication on our neuropsychological findings. During the 3rd year of the grant we will continue to analyze neuropsychological findings, as well as continue to collect and analyze MRSI and fMRI data; we also will prepare manuscripts linking neuropsychological and MRSI/fMRI findings.

Number of Patients Seen: During the second year of the grant, we have seen 31 patients altogether, four with NF-1, 13 without NF-1, and 13 children with a reading disability (RD); one of the four patients with NF-1 was a sibling pair. One patient with NF-1 did not meet criteria for being included in the study because the results of the MRI determined that he was ineligible. Two children in the control group were found to be ineligible due to psychiatric issues. Three children with RD were determined ineligible for the study: two because of reading scores that fell between the 25th and 40th percentile (thus meeting neither RD or control criteria) and one had an IQ below 80. For those children who were found to be ineligible for the study, it did not present a problem because all of them participated fully in the testing and the parents received appropriate feedback (i.e., they will not be included in data analyses). Below is a chart of the participants:

	1ST YEAR	2nd YEAR	GOAL OVER 3 YRS	% OF TOTAL
NF-1 W/OUT SIBLING	6	3	20	65%
NF-1 W/SIBLING	3	1	10	40%
NF-1 SIBLING (NON AFFECTED)	3	1	10	40%
CONTROLS	5	13	20	90%
READING DISABILITIES	1	13	30	47%
TOTAL NUMBER SEEN:	**18**	**31**	**95**	**52%**

Preliminary Findings/Progress:

MRI Findings: Data from the MRSI scans that we have collected have been analyzed by Dr. Barker's group. For the fMRI component of the grant, we have developed and piloted the paradigms for both the visuospatial and phonological (reading) fMRI tasks, and have collected data on 5 patients. Below are results for the visuospatial task (analogous to the Judgment of Line Orientation). **Figure 1** depicts activation for the visuospatial task in a 12 year old female with NF-1. Activation is seen bilaterally in the occipital lobe, more right-than-left.
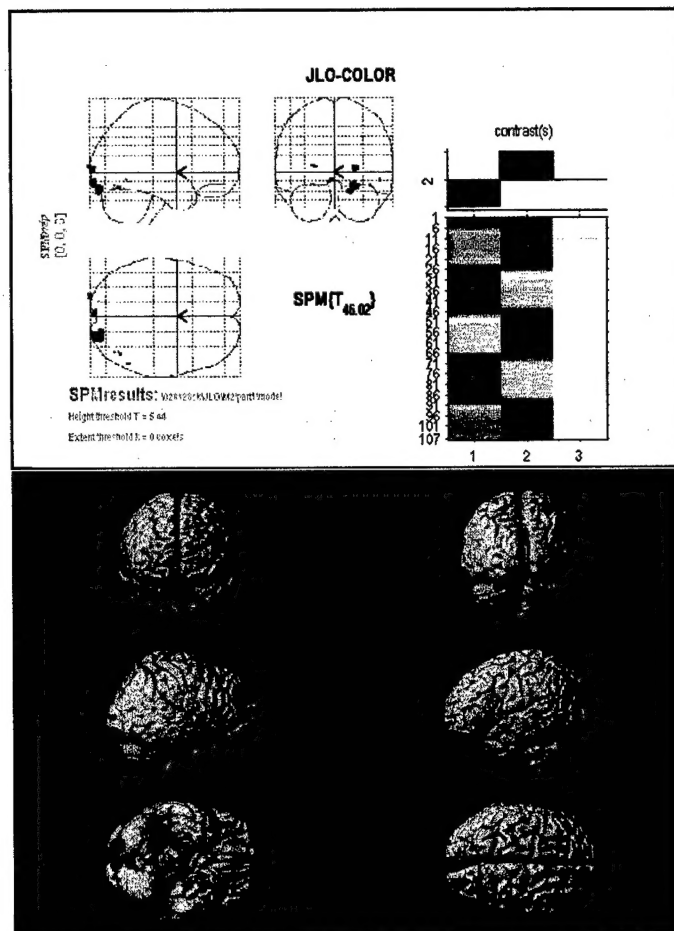
Figure 1 Single-subject data from a 12-year old female with NF-1 for the visuospatial task. Each statistical parametric map shows JLO *vs.* Color, where activation was more significant for a block of deciding line orientation than for a block judging whether two sets of lines were the same color. Activation is shown at corrected p < 0.05 (female, age 12). Bilateral occipital activation, more right than left, is observed.

Preliminary Neuropsychological Findings: Data analyses examining children with NF-1, controls, and children with RD are presented below; we do not yet have enough data from the MRI components of the grant to present integrated neuropsychological and MRI findings (this is our primary goal for next year). Preliminary analyses on neuropsychological data comparing children with NF-1, children with RD, and Controls (we excluded siblings from analyses) on the language and reading measures suggest that children with NF-1 show similar deficits to children with RD. Multiple Analyses of Variance (MANOVAs) suggest that children with NF-1 have similar difficulties as children with RD, with both groups showing weaknesses in reading accuracy (decoding), reading comprehension, receptive language, and figurative language. On the other hand, children with NF-1 appear to have some notable differences from children with RD. Unlike children with RD, results suggest that immediate memory, expressive language, inferential language, phonological memory, rate of retrieval, as well as reading rate are relatively spared in children with NF-1. If these findings prove to be true, intervention

for the associated learning disabilities in children with NF-1 will be able to be tailored to this pattern of strengths and weaknesses (e.g., strong inferential abilities may help remediate language disabilities). Preliminary results are listed in Table 1:

Table 1

TEST / Subtest	NF-1 vs. Control (p-values)	RD vs. Control (p-values)	NF-1 vs. RD (p-values)
Clinical Evaluation of Language Fundamentals – 3 (CELF-3); Receptive Language	.008	.005	n.s.
CELF-3; Expressive Language	n.s.	.001	n.s.
Test of Language Competence (TLC); Ambiguous Sentences	n.s.	.003	n.s.
TLC; Figurative Language	.024	.001	n.s.
TLC; Inferences	n.s.	.026	n.s.
Gray Oral Reading Test – 3 (GORT-3); Reading Accuracy	.006	.001	n.s.
GORT-3; Reading Rate	n.s.	.001	.013
GORT-3 Comprehension	n.s.	.001	.018
Wide Range Test of Memory and Learning (WRAML); Immediate Recall	n.s.	.009	n.s.
Weschler Individual Achievement Test (WIAT); Basic Reading	.001	.001	.003
WIAT; Reading Comprehension	.007	.001	n.s.
WIAT; Listening Comprehension	n.s.	n.s.	n.s.
Comprehensive Test of Phonological Processing (CTOPP); Phonological Awareness	n.s.	n.s.	n.s.
CTOPP; Phonological Memory	n.s.	.017	n.s.
CTOPP; Rapid Automatized Naming	n.s.	.003	n.s.

Problems in Accomplishing Tasks: This year we have had no problems accomplishing tasks. In contrast to the first year of the grant, for which we had significant "external" impediments that did not allowed us to recruit and evaluate subjects for a total six months (which were: final approval from the US Army Medical Research and Material Command Human Subjects board and shut down of all research at Johns Hopkins Medical Institutions mandated by the Office for Human Research Protections), the second year of the grant has gone smoothly. This year we have successfully recruited and tested 31 children. We anticipate that our final year of the grant will be highly productive; as the "word is getting out" about the project, we have increasingly more families calling to participate. Thus, we anticipate a highly productive final year of the grant.

Recommended Changes: So far, we have not encountered any issues that suggest that we should consider changing our goals/procedures of the grant in any manner. We have not

encountered any significant obstacles in our research during the second year, and we anticipate a highly productive final year of the grant.

KEY RESEARCH ACCOMPLISHMENTS

➢ Have identified and established connections with many recruiting sources
➢ Have seen 49 subjects:
   o 4 sibling pairs
➢ Have entered all neuropsychological data and conducted statistical analyses.
➢ Have an accepted abstract to be presented at the *International Neuropsychological Society* meeting in February.
➢ Have a manuscript in preparation regarding the neuropsychological findings between NF-1, RD, and Control groups.
➢ Have collected and analyzed MRSI data for 4 children with NF-1 (from sibling pairs)
➢ Developed and piloted both fMRI paradigms
➢ Acquired and analyzed data on 5 patients using fMRI visuospatial and phonological (reading) tasks.

REPORTABLE OUTCOMES

There are several reportable outcomes that have resulted directly from this grant. First, we have an accepted abstract on the neuropsychological findings that we will be presenting at the *International Neuropsychological Meeting* in February in Honolulu, Hawaii. Findings showed that both the NF-1 and RD groups showed lower scores that the control group on measures of reading and language, although the NF-1 group performed higher than the RD group on the reading measures. Unlike children with RD, children with NF-1 did not show impairment on Rapid Automatized Naming, which tends to be predictive of reading fluency. Second, while not directly resulting from the data collected from this grant, this grant has helped support our overall program of research on NF-1 at the Kennedy Krieger Institute. This includes one accepted abstract and three publications (see Appendix). One publication, currently in press in *Neurology*, provides detailed analyses of cortical gray and white matter volumes in males with NF-1; lobar (frontal, occipital, parietal, and temporal lobes) and lobar subdivisions (e.g., prefrontal lobe) areas were measured. Findings showed increase in frontal and parietal white matter volumes in patients with NF-1, and frontal gray matter reductions in males with NF-1 who also had ADHD. Another publication, published in the *Journal of the International Neuropsychological Society*, examined the growth of "spared" and "impaired" cognitive measures in children with NF-1 as compared to their siblings. Findings indicated that over time children with NF-1 do not "catch up" to their siblings on those measures that were "impaired"; furthermore, there were no significant differences in growth rates between children with NF-1 and their siblings for the "spared" and "impaired" cognitive functions. We also have examined the longitudinal evolution of T2-weighted hyperintensities (UBOs; manuscript currently under revision for the *American Journal of Medical Genetics*). Findings showed that the total number of UBO-occupied locations

evolved in a non-linear manner, with a decrease between approximately ages 7-12 years, followed by a progressive increase in adolescence. The same pattern was also found for UBO number and/or volume for all regions, with the exception of cerebellar hemispheres. Finally, we have an accepted abstract linking the neuropsychological and volumetric MRI findings that we will be presenting at the *International Neuropsychological Meeting* in February in Honolulu, Hawaii. Findings showed an inverse relationship between frontal lobe volumes and the Judgment of Line Orientation test, regardless of group membership.

We have applied for funding from the Department of the Army, US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND, to continue our work towards understanding the neurological correlates of the language and reading disabilities reported in children with NF-1. Specifically, we are focusing on refining the knowledge of how to treat children with NF-1 who have reading disabilities as compared to children with idiopathic reading disabilities (IRD). For the first goal of this research, we propose to continue our research using MRSI and neuropsychological testing to examine the risk factors associated with having a reading/language disability in NF-1. The second goal of the research is to determine if children with NF-1 who have reading disabilities respond in the same manner, both neurobiologically (by use of fMRI) and neuropsychologically, to educational interventions known to be highly effective for children with IRD. It is has been established that specialized educational interventions are highly successful for children with IRD, resulting in not only improved reading abilities, but also "normalization" brain activation during reading tasks (using fMRI). We seek to determine if these same interventions will be as effective for children with NF-1.

We have applied for funding from the National Institutes of Health (NIH; R01 HD 044073-01), "Cognitive and Neural Mechanisms of Reading Comprehension". This grant that has been applied for relates to our understanding of idiopathic reading and language disorders, which is relevant to treating the reading and language disorders prevalent in NF-1.

CONCLUSIONS

We have had significant success in reaching the goals of the grant this year. We have seen 31 children and are close to our targeted numbers of enrollment at this point of our grant. We have also developed our fMRI paradigms and have collected and analyzed fMRI data on five children. Additionally, we have analyzed MRSI data on the 4 sibling pairs that we have seen. Thus, despite some of our setbacks during the first year of the grant (our inability to recruit and evaluate subjects for six months of the first year of the grant because of waiting for human subjects approval), during the second year of the grant we have been able to "catch up" in terms of progress on the grant. We have now addressed many of the goals of the grant. Preliminary findings suggest that children with NF-1 show similar difficulties as children with RD, with both groups showing weaknesses in reading accuracy (decoding), reading comprehension, receptive language, and figurative language. On the other hand, children with NF-1 appear to have some notable differences from children with RD. Unlike children with RD, results suggest that

immediate memory, expressive language, inferential language, phonological memory, rate of retrieval, as well as reading rate are relatively spared in children with NF-1. If these findings prove to be true, intervention for the associated learning disabilities in children with NF-1 will be able to be tailored to this pattern of strengths and weaknesses (e.g., strong inferential abilities may help remediate language disabilities). This grant has also helped support our overall program of research on NF-1 at the Kennedy Krieger Institute. This includes three publications (one published in *Journal of the International Neuropsychological Society*, one in press in *Neurology* and another under revision in the *American Journal of Medical Genetics*) and two accepted abstracts to be presented at the International Neuropsychological Society meeting in February 2003.

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None.

APPENDICES

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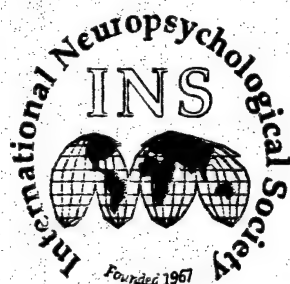
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Cutting, LE, Cooper, KL, Koth, CW, Mostofsky, SH, Kates, WR, Denckla MB, & Kaufmann, W.E. (in press). Megalencephaly in NF1: Predominantly White Matter Contribution and Mitigation by ADHD. *Neurology.*

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Thirty-First Annual Meeting   February 5-8, 2003
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Authors: L.E. Cutting, C.W. Koth, D. David, & M. Denckla

Title: **Comparison of Neuropsychological Profiles of Children with NF-1 and RD**

Text: It has been reported that children with Neurofibromatosis Type 1 (NF-1) have a high incidence of learning disabilities, particularly reading disabilities (RD). However, it is not fully understood whether there are differences in neuropsychological performance between children with NF-1 versus children with idiopathic RD, which might be important in determining the best instructional/intervention techniques for children with NF-1. Ten children with NF-1, 9 children with idiopathic RD, and 10 children without RD or NF-1 (Controls) were compared on reading and language measures using univariate and multivariate analyses of variance. Results indicated that the RD group showed lower scores than the NF-1 group on measures of single word reading and reading comprehension ($p<.001$); however, both groups' performance on these measures was below that of the control group's ($p<.001$). Both NF-1 and RD groups showed significantly lower scores than the control group on measures of phonological awareness and phonological memory (all $p<.01$); however, unlike the RD group, the NF-1 did not show impairment on Rapid Automatized Naming ($p>.05$), which tends to be predictive of single word reading fluency. On language measures, the RD and NF-1 groups showed significantly lower performance than the control group on receptive and expressive language, as well as inferential and figurative language, even when scores were adjusted for IQ (all $p<.05$). Overall, findings indicate that children with NF-1 show similar neuropsychological performance to that of children with idiopathic RD, suggesting that similar types of instructional/intervention techniques known to be successful with children with RD may be beneficial for children with NF-1.

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Title: Relationship between parietal and frontal lobe volumes and cognitive functioning in NF-1

Text

Studies have shown selective cognitive impairment and megalencephaly to be prominent characteristics of Neurofibromatosis Type 1 (NF-1); however, the relationship between brain tissue volume and cognition remains uncertain. In order to clarify this issue, we compared brain volumes and neuropsychological functioning in 18 males with NF-1 and 16 controls, aged 5 -18 years. Using the Talairach parcellation technique, parietal and frontal lobe tissue volumes were measured. These volumes were then compared to performance on a visuospatial test traditionally assumed to reflect parietal lobe function (Judgement of Line Orientation; JLO). Consistent with previous findings, the NF-1 group scored significantly lower on the JLO ($p<.001$), and had larger total cerebral, parietal, and frontal lobe volumes as compared to controls (all $p<.050$). Multiple-regression analyses, controlling for age and total cerebral volume, were utilized to examine the *relationship* between brain volumes and neuropsychological performance. Results revealed that, regardless of group membership, there was a significant inverse relationship between frontal lobe volumes and JLO ($p=.008$) performance, whereas the specific relationship between JLO performance and parietal lobe volume was not significant. This suggests that in pediatric populations, JLO performance may be influenced by cognitive functions typically associated with the frontal lobe (i.e., executive functioning).

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Growth curve analyses of neuropsychological profiles in children with neurofibromatosis Type 1: Specific cognitive tests remain "Spared" and "Impaired" over time

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Abstract

Cognitive deficits in neurofibromatosis Type 1 (NF-1) have been documented in both the verbal and visuospatial domains. Previous investigations from our laboratory have determined a specific pattern of "spared" (Picture Arrangement, Picture Completion, and Rapid Automatized Naming) and "impaired" (Judgment of Line Orientation, Vocabulary, and Block Design) performance on cognitive measures in this population when compared to sibling-matched controls in pairwise designs. Growth curve analyses were conducted on these repeated measures in 19 patients with NF-1 and their siblings to investigate the longitudinal course and growth pattern of these spared and impaired measures. Results indicated that over time children with NF-1 do not catch up to their siblings on impaired measures, and they continue to perform similarly to their siblings on the spared measures. With respect to growth rates, on average across the 6 cognitive measures there was no significant difference between the groups. However, the variation *among* families for level of performance was estimated to be larger than variation among siblings within a family for 2 out of 6 cognitive measures (i.e., providing for these 2, Vocabulary and Rapid Automatized Naming, evidence of substantial familial correlation), suggesting that there is need to consider NF-1 associated deficits within a familial context. (*JINS*, 2002, *8*, 838–846.)

Keywords: Genetics, Cognition, Longitudinal analyses

INTRODUCTION

Neurofibromatosis Type 1 (NF–1) is a common autosomal dominant genetic disorder with an incidence of 1:4000 in the population (Huson, 1989, 1994; Stumpf et al., 1988). About half of the NF-1 cases are sporadic, *versus* familial, in nature. National Institutes of Health Consenses Conference diagnostic criteria, two or more of which must be present for diagnosis of NF-1, include *café au lait* macules, nerve tumors within or below the skin, Lisch nodules, optic glioma, a bony lesion, freckling in armpit or groin area, and/or a first degree relative with NF-1. Other neurological signs/symptoms of NF-1 that are not currently within the diagnostic criteria are megalencephaly, T2 weighted hyperintensities (unidentified bright signals seen on magnetic resonance imaging scans; UBS), and elevated *N*-acetylaspartate/choline ratio in the thalamus (Cutting et al., 2000a; Denckla et al., 1996; North et al., 1997; Wang et al., 2000). In addition to these neurological abnormalities, the NF-1 gene appears to have an impact on cognition; specifically, there is a much higher prevalence rate of learning disabilities in the NF-1 population than in the general population (30–65% *vs.* 5–17.5%; North et al., 1997; Riccardi, 1981; Shaywitz & Shaywitz, 1999).

While it was originally thought that the type of learning disability associated with NF-1 was nonverbal, or visuospatial, in nature (Eliason, 1986), recent investigations have established that children and adolescents with NF-1 have

reading and language deficits. These language and reading deficits are much more dramatic than their visuospatial/ nonverbal deficits (e.g., Brewer et al., 1997; Mazzocco et al., 1995) and are similar to those children in the general population who have reading disabilities (Cutting et al., 2000b). However, children with NF-1 are differentiated from children in the general population who have reading disabilities by the presence of additional deficits in broad language as well as visuospatial areas (Cutting et al., 2000b).

Another distinctive feature that has been observed in our laboratory in the domain of cognition in NF-1 is the presence of a pattern of "sparing" and "impairing" on certain neuropsychological tests that represent specific cognitive functions in the verbal and nonverbal domain (see Table 2). Previous studies from our laboratory (Hofman et al., 1994; Mazzocco et al., 1995), which have used a sibling matched pair design (see below for discussion of sibling pair matched designs *vs.* randomly selected control group designs), have established that despite the reading, language, and visuospatial deficits in NF-1, there appears to be a consistent pattern of impaired and spared tests across the verbal and nonverbal domains. Within the verbal domain, Rapid Automatized Naming (Denckla & Rudel, 1976) is spared, while Vocabulary (Wechsler, 1974, 1991) is impaired. Within the nonverbal domain, Picture Completion and Picture Arrangement are spared, while Block Design and Judgment of Line Orientation (JLO; Benton et al., 1983) are impaired. Picture Completion and Picture Arrangement are labeled as nonverbal tests because they don't require a verbal response; however, it should be noted that these tasks do imply covert language components (such as, unless the person chooses to point, word retrieval for Picture Completion, and demands for inner language as story narrative for guiding the card sequencing in Picture Arrangement). On the other hand, Block Design and the JLO, which are also considered to be within the nonverbal domain, have fewer implicit word retrieval and inner language demands; thus, they are more likely to be representative of visuospatial ability. It should be noted that exactly *why* children with NF-1 exhibit this distinctive pattern of performance is not clear at this time, as the pattern was derived *empirically* and did not originate from theoretically driven hypotheses. Thus, the explanation behind this distinctive pattern on these measures in children with NF-1, as well as replication of the pattern across laboratories, awaits further study.

Even though this pattern of spared and impaired performance on neuropsychological tests is known to be present in children with NF-1, there have been no investigations of the growth patterns of these cognitive tests within a familial, or sibling pair-wise, design. Thus, it is not known whether performance on these tests remain, respectively, impaired or spared over time. Growth curve analysis provides a way to take into account both continued *absolute lowering* (i.e., impairment) over time as well as possibility of abnormal *patterns of growth* in children with NF-1. Previous investigations (Hofman et al., 1994; Mazzocco et al., 1995) conducted in our Center have utilized a sibling matched pair

design. A sibling matched pair design, unlike that which involves a control group from the general population, takes into account familial and environmental factors (Mackintosh, 1998), thus allowing for a clearer determination of the impact of the NF-1 gene on cognition. In this present investigation, we used the sibling design because of our desire to specifically investigate the impact of the NF-1 gene on cognition over time, as well as because of some associated specific areas/questions of interest, which were as follows:

- What is the relative size of variation among siblings within a family as compared to variation among families? This question is important because it addresses the fundamental issue of whether the sibling-matched pair design *is* really essential when studying the impact of a genetic disorder on cognition.

- Do the absolute differences between NF-1 and their siblings on impaired tests remain significant over time? Do the absolute differences between NF-1 and their siblings on spared tests remain nonsignificant over time?

- What are the growth patterns, or developmental trajectories, for these cognitive tests in children/adolescents? Are they different in children with NF-1 *versus* their siblings?

MATERIALS AND METHODS

Research Participants

Children/adolescents in the NF-1 group were originally included in the study if they were between 6 and 16 years old, had received a diagnosis of NF-1, and had an unaffected sibling (or siblings) also between the ages of 6 and 16 years old. Both the child/adolescent with NF-1 and his/her sibling could have no other known neurological disorder that could contribute to having a learning disability. Specific exclusionary criteria for children with NF-1 were presence of optic gliomas and/or other brain tumors. Subjects were recruited from a variety of sources, such as NF-1 clinics, newsletters, and national organizations. (Please see Hofman et al., 1994; Mazzocco et al., 1995 for full descriptions of recruitment procedures and inclusionary/exclusionary criteria.) Since the beginning of the study in 1989, approximately 35 NF-1/sibling pairs have participated in the LDRC project. Participation in the LDRC project includes 1 day of comprehensive psychoeducational (IQ and achievement) and neuropsychological (visuospatial, visual–motor, language, working memory, reading, and reading-related) tests in addition to another day of neuroimaging (a structural magnetic resonance imaging scan) and other neurological tests (e.g., oculomotor testing). The study was approved by the local IRB committee and informed consent (and assent) was obtained for all subjects participating in the study.

The 35 NF-1/sibling pairs who originally participated in the LDRC project were invited to participate in the longitudinal component of the study. To date, approximately 19 families have expressed interest in participating in the lon-

Table 1. Descriptive statistics for NF-1 and sibling groups

Variable	NF-1	Siblings
Mean age and age ranges[1]	9.16 (2.32; range 6–13)	9.14 (2.59; range 6–14)
Mean IQ and IQ ranges[1]	98.74 (12.20; range 80–127)	110.71 (12.13; range 89–136)
Gender	16 males, 3 females	13 males, 8 females

[1]*Note.* Age ranges and mean IQ and IQ ranges are from the initial visit.

gitudinal component of the study; of these 19 NF-1 sibling pairs (note that two "pairs" actually were triplets and had 2 nonaffected siblings and 1 child with NF-1), 7 have been seen only once, 2 have been seen twice, and 10 have been seen between three and five times (see Analyses section for the rationale as to why we included subjects seen less than three times). Subjects initially completed the comprehensive 2 days of cognitive and neurological testing described above; thereafter, every alternate year the comprehensive cognitive battery was re-administered, with between-year visits consisting of an abbreviated battery of tests (which included the impaired and spared tests). Socioeconomic status (SES) was estimated by the Hollingshead (1975); the mean Hollingshead score for 18 of the 19 families (1 family did not complete the Hollingshead questionnaire) was 49.56 (*SD* = 10.58), with 8 of the families in the highest SES category (Level I), 7 scoring at Level II, 2 at Level III, and 1 at Level IV. The racial distribution of the group was predominately White (17 of the 19 pairs), with only 2 non-White families, 1 African American and the other biracial, in the group. Mean age and age ranges and mean IQ (and IQ ranges) at the initial visit, as well as gender distribution, for children with NF-1 and their siblings are listed in Table 1.

Neuropsychological Measures

Neuropsychological measures were selected based upon previous findings (Mazzocco et al., 1995) that NF-1 is associated with a certain pattern of sparing and impairing on specific tests; a brief description of each measure and its spared or impaired status is provided in Table 2. All analyses were conducted using raw scores (age was accounted for in the statistical model; see Analyses section below). For the four subtests (Vocabulary, Picture Arrangement, Picture Completion, and Block Design) that were administered from the Wechsler Intelligence Scales (Wechsler, 1974, 1981, 1991), one of three tests/versions were administered, depending on the age of the child/adolescent and/or when the subject participated in the study (i.e., the first phase of the LDRC, 1989 to 1994 or the second phase of the LDRC, 1995 to 2000). The versions of the Wechsler Intelligence Scales utilized were (1) the Wechsler Intelligence Scales for Children–Revised (WISC–R; Wechsler, 1974; for children younger than 17 years who were seen any time from 1989 to 1994); (2) Wechsler Intelligence Scales for Children–Third Edition (WISC–III; Wechsler, 1991; for children younger than 17 years who were seen after 1994); or (3) Wechsler

Adult Intelligence Scales–Revised (WAIS–R; Wechsler, 1981; for adolescents 17 and older and adults). The four subtests are very similar from all three Wechsler Intelligence Scales; however, any possible effects of using different versions were accounted for statistically (see Analysis section).

Analyses

Growth curve analyses were conducted on the cognitive tests in patients with NF-1 and their siblings in order to investigate the longitudinal nature and the growth pattern of these spared and impaired tests. All 19 NF-1/sibling pairs were used in analyses; subjects who had missing data points (i.e., were unable to complete certain cognitive tests/

Table 2. Description of "impaired" and "spared" neuropsychological tests

Tests	Description
"Spared" tests	
Rapid Automatized Naming	The ability to name quickly within a well learned restricted category of visual stimuli (e.g., letters and numbers)
Picture Completion	Attention to and recognition of missing visual details in pictures
Picture Arrangement	A visual sequencing and language related task involving the sequencing of pictures to tell a story
"Impaired" Tests	
Block Design	Timed task of assembling blocks to replicate a two-dimensional geometric model
Vocabulary	Word knowledge and oral expression; requires formulating definitions of words
JLO	Requires one to determine the orientation of two lines from 11 different possible orientations

Note. JLO = Judgment of Line Orientation.

subtests because of testing was discontinued due to fatigue or extreme difficulty with the test) were excluded for the particular analysis with that cognitive test. Two siblings and 1 child with NF-1 did not receive Picture Completion on their third visits; 1 sibling did not receive Rapid Automatized Naming on his/her second visit; 2 children with NF-1 did not receive the JLO on their first visit; and 1 child did not receive the JLO on his/her second visit. We used random effects regression models to describe the relationship of each cognitive score with age, NF-1 status (either affected or unaffected), test type (WISC–R, WISC–III, WAIS–R), and gender. A random effects model was used, rather than traditional linear regression, for several reasons. Linear regression ignores the association among measurements from the same child and the association among measurements from the same family (correlation of observations of cognitive tests from the same child arises because of the heterogeneity among children and families in their true growth curves) and would therefore yield inefficient parameter estimates and incorrect inferences (Liang & Zeger, 1993). In addition, children also entered the study at different baseline cognitive scores and therefore would be likely to have different growth rates. A random effects model is therefore a reasonable description of the data if collection of baseline cognitive scores and growth rates can be thought of as sampling from a distribution across families and children.

The random effects model[1], fitted separately for each of the cognitive tests, was as follows: $Y_{ijk} = (B_0 + b_{0ij}) + [(B_1 + b_{1i})(age_{ijk} - 14)] + B_2(age_{ijk} - 14)^2 + B_3 NF\text{-}1_{ijk} + B_4 Test1_{ijk} + B_5 Test2_{ijk} + B_6 gender_{ijk} + B_7[(age_{ijk} - 14) \times NF\text{-}1_{ijk}] + \epsilon_{ijk}$

- age_{ijk} = the age of ith family's jth child at kth visit (Note: age was centered at 14 years to avoid collinearality)

- $NF\text{-}1_{ijk}$ = 1 if the child had NF-1 and 0 if the child did not have NF-1

- $Test1_{ijk}$ = 1 if the test was the WISC–R or 0 if not

- $Test2_{ijk}$ = 1 if the test was the WISC–III or 0 if not

- $Gender_{ijk}$ = 1 if the child was a boy and 0 if the child was a girl

In the model, Y_{ijk} represents the cognitive scores for the ith family, the jth child, and the kth measurement. $B_0, B_1 \ldots B_7$ are fixed effects, which are constant; b_{0ij} and b_{1i} are random effects, which follow a bivariate normal distribution. The above random effects model has two important features. First, based on the exploratory data analysis (not shown), we assumed that the growth rates between a child/adolescent with NF-1 and his/her unaffected sibling were the same across families. Second, we used all 19 sibling

[1]Note that test type predictors were not included in the model for Rapid Automatized Naming and JLO cognitive tests.

pairs in the analysis, even when only 10 of them had three or more visits. The rationale for this is that children/adolescents with less than three visits still inform us about the differences in the cognitive scores at the earlier visits. They also provide useful information about the variability across people and over time in the cognitive scores.

RESULTS

From the results in Table 3 the following can be seen:

1. There were no significant differences between males and females on any cognitive measure.

2. Subjects scored higher on WISC–R subtests than WAIS–R subtests for Vocabulary, Block Design, Picture Arrangement, and Picture Completion; subjects scored higher on WISC–III subtests than WAIS–R subtests for Block Design, Picture Arrangement, and Picture Completion; subjects scored higher on the WISC–R Vocabulary and Block Design subtests than on WISC–III Vocabulary and Block Design subtests. (Note that this suggests that there were significant differences between the different versions of the tests; however, having these terms in the

Table 3. *P*-values for terms in the model each cognitive test

Effect	Estimate	Standard error	*P*-value
Test			
Vocabulary			
(age − 14)	1.9198	.3268	.0001
$(age_{ijk} - 14)^2$	−.1842	.0476	.0026
NF-1 status	−6.6336	1.4130	.0001
$(age_{ijk} - 14) \times$ NF-1 Status	−.1084	.2895	.7101
Block Design			
(age − 14)	3.0668	.4638	.0001
$(age_{ijk} - 14)^2$	−.1237	.0644	.0810
NF-1 status	−12.5644	3.6898	.0015
$(age_{ijk} - 14) \times$ NF-1 Status	−.7302	.4661	.1251
JLO			
(age − 14)	.5524	.2020	.0194
$(age_{ijk} - 14)^2$	−.1194	.0303	.0023
NF-1 status	−4.7290	1.3368	.0010
$(age_{ijk} - 14) \times$ NF-1 Status	.3602	.2459	.1505
Picture Arrangement			
(age − 14)	2.0746	.4904	.0014
$(age_{ijk} - 14)^2$	−.2142	.0758	.0165
NF-1 status	−1.6420	2.7929	.5599
$(age_{ijk} - 14) \times$ NF-1 Status	.1262	.5033	.8032
Picture Completion			
(age − 14)	1.0643	.2185	.0005
$(age_{ijk} - 14)^2$	−.1030	.0342	.0118
NF-1 status	−1.0060	.8786	.2596
$(age_{ijk} - 14) \times$ NF-1 Status	−.2050	.1951	.3002
Rapid Automatized Naming			
(age − 14)	−1.5702	.3843	.0018
$(age_{ijk} - 14)^2$	.3002	.0475	.0001
NF-1 status	.6032	1.7255	.7284
$(age_{ijk} - 14) \times$ NF-1 Status	.2014	.3301	.5452

Note. JLO = Judgment of Line Orientation.

model essentially covaries, or controls, the influence of the different tests on the rest of results.)

3. The linear age term in the model, $(B_1 + b_{1i})(\text{age}_{ijk} - 14)$ was significant for all cognitive tests, suggesting, not surprisingly, that over time there is growth in these cognitive tests.

4. The quadratic age term, $B_2(\text{age}_{ijk} - 14)^2$, was significant for all cognitive tests *except* Block Design, suggesting (also not surprisingly) that over time there is a tendency for these tests to begin to level out in their growth. Note that there was a trend toward significance for the quadratic term for Block Design ($p = .0810$).

5. The coefficient for $(\text{age}_{ijk} - 14) \times \text{NF-1}_{ijk}$ was not significant for all six cognitive tests; therefore, on average, NF-1 children and their siblings show the same growth rates across these six tests.

6. Children/adolescents with NF-1 scored significantly lower on Vocabulary, Block Design, and JLO ($p = .0001$, $p = .0015$ $p = .0010$, respectively) than their unaffected siblings (see Figure 1).

7. Children/adolescents with NF-1 did not score significantly lower than their siblings on Picture Arrangement, Picture Completion, and Rapid Automatized Naming ($p = .5599, .2596, .7284$, respectively; see Figure 2).

Other results, concerning families' level of performance and growth rates, were obtained by examining results of b_{1i} in the random effects model (shown above). Ideally, we seek to estimate the variation in the level (intercept) and trend (slope) in cognitive functioning measures among children within and across families. For the level of functioning, there would be two variance components, the first describing differences among siblings within a family, σ_1^2, and the second quantifying differences among families, σ_2^2. The correlation of siblings is then given by $\sigma_1^2/(\sigma_1^2 + \sigma_2^2)$.

We attempted to estimate a model that allows for variation across children in both the level and trend in functioning. However, there was insufficient evidence in the data set. Hence, we have estimated the two variance components for only the level of functioning. Table 3 shows the results for each of the six measures.

For Picture Completion, Picture Arrangement, Block Design, and Judgment of Line Orientation, we estimate that the variation between siblings from a given family is substantially larger than the differences among families. For these variables, the correlation among siblings in the level of functioning will be small. That is, siblings do not appear to be more similar in level of performance to each other than unrelated children. However, for Vocabulary and Rapid Automatized Naming, the estimated variation among families is relatively larger, providing evidence of greater familial correlation. Caution in interpreting these patterns is warranted as the data set is small and the variance components are not well determined because of large confidence intervals.

DISCUSSION

This study was conducted to examine the patterns of growth on certain cognitive measures over time in children with NF-1, as compared to their unaffected siblings. In terms of overall growth in all children (both NF-1 and siblings), not surprisingly, there were increases in performance on all the cognitive measures over time; in addition, for all the cognitive measures except Block Design (which showed a trend towards significance), there was a gradual leveling off of growth, thus suggesting that children have a tendency to gain more at an earlier age and that growth is not as rapid as children/adolescents get older. In terms of differences between children/adolescents with NF-1 and their siblings, results indicated that those cognitive tests that were spared remained so over time, as did those tests that were impaired, thus suggesting that the profile of spared and impaired tests is stable, with a specific pattern of cognitive strengths and deficits characterizing NF-1 over the long term. However, it was not only of interest to determine whether these cognitive tests remained stable in their spared/impaired status, but also whether children with NF-1 had different patterns, or trajectories, of growth in these tests as compared to their siblings. Results indicated that over time, children with NF-1 do *not* appear to have patterns of growth different from their siblings on these cognitive tests. Thus, while children/adolescents with NF-1 continue to have lower scores as they get older, their pattern of growth is similar to that of their siblings. (In other words, if thought of mathematically, the graph of cognitive scores for children with NF-1 and their siblings are parallel in slope, but the lines for the scores for children/adolescents with NF-1 are lower in their y-intercept value than those of their siblings.) Finally, it was found that there is evidence for greater familial correlation than for unrelated children for the level of performance on two out of the six cognitive tests.

Because children/adolescents with NF-1 continue to have this certain spared/impaired cognitive pattern without a deviant pattern of growth, there are potential clinical implications from the findings of this study. It may be useful to monitor and/or provide early intervention within certain areas of cognitive development in children/adolescents with NF-1; therefore, providing opportunities for vocabulary enrichment as well as guided experience and practice with visuospatial material early may be worthy of consideration to decrease the chances of academic difficulties and/or frustrations arising. Likewise, early identification of areas of strength is an important consideration so that these areas can be capitalized on and used to compensate for weaknesses throughout cognitive development. Of course, it should be kept in mind that even though a score represents a weakness within a child's cognitive profile and within a genetic/familial context, it may *not* be below average when compared with national norms; however, the presence of a *relative* deficit could instigate issues of frustration and low self-esteem with regard to academics.
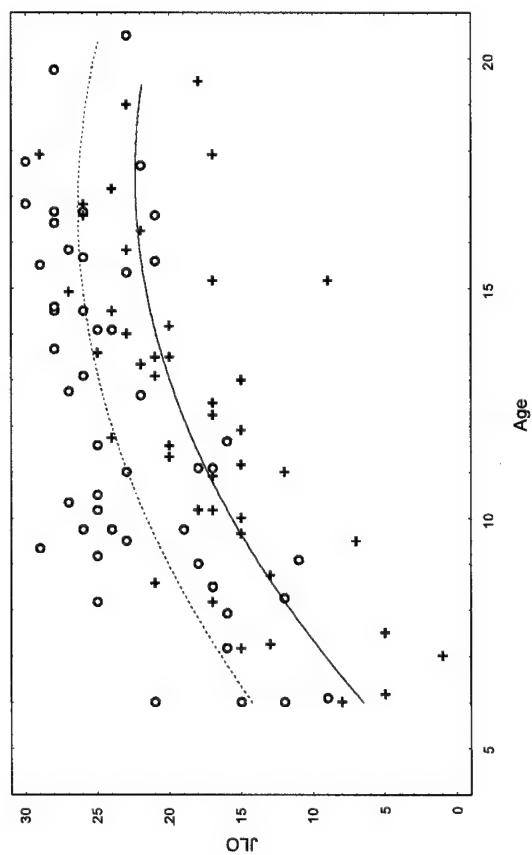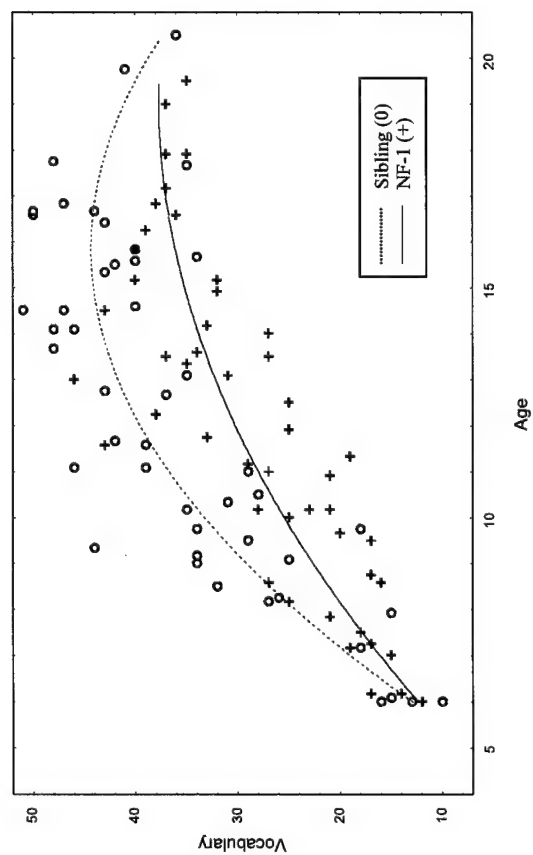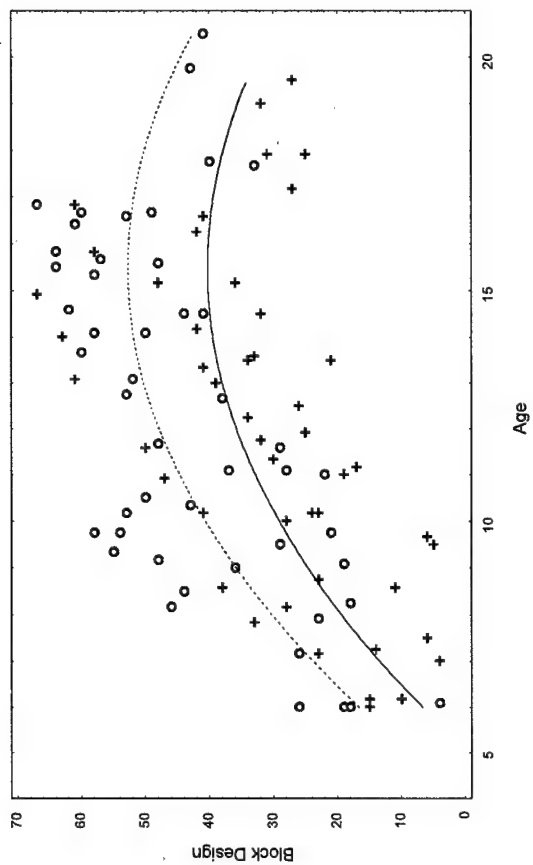
Fig. 1. Growth curves for "impaired" functions
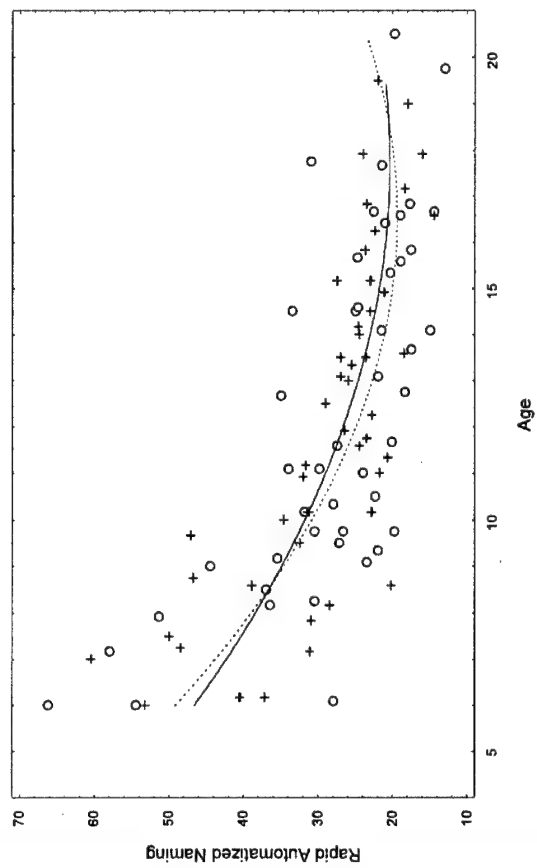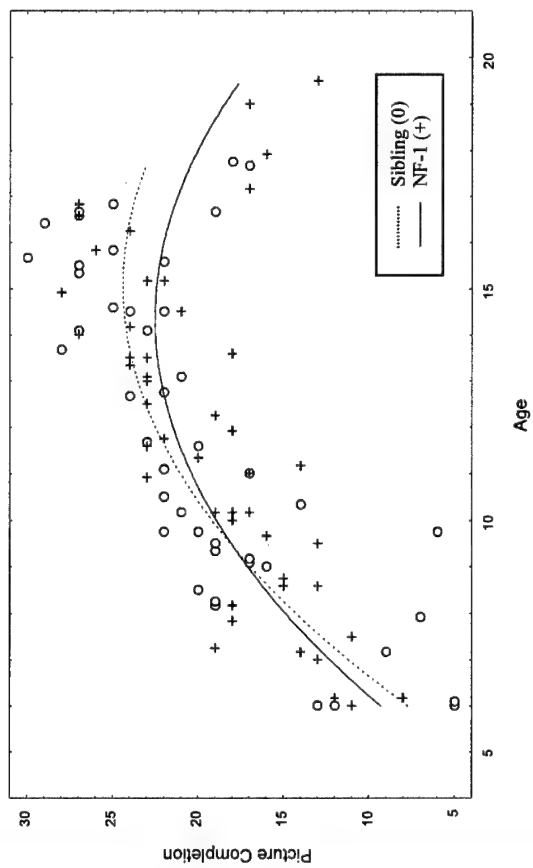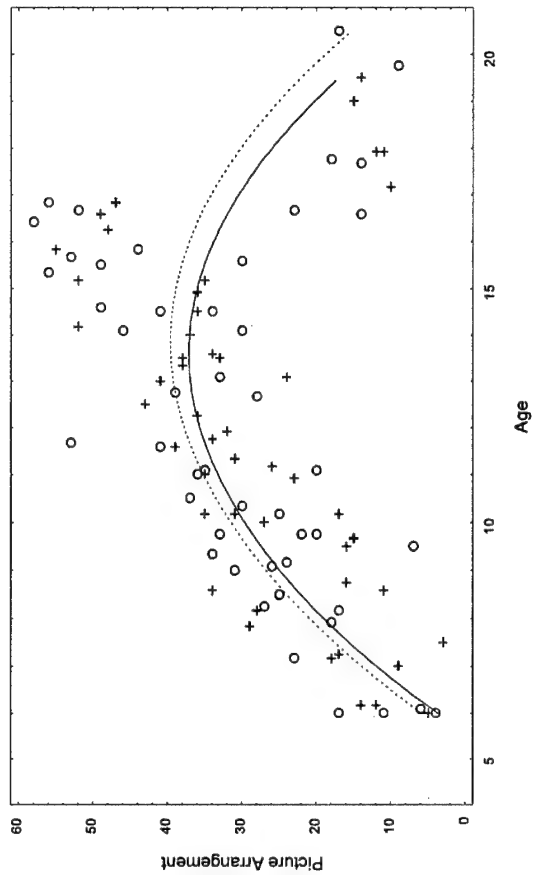
Note: JLO = Judgment of Line Orientation

Fig. 2. Growth curves for "spared" functions

An important implication of this study for future studies of cognitive deficits in genetic disorders is the finding that for *certain* cognitive tests, there is evidence for greater familial correlation than for unrelated children for the level of performance. Exactly why we found evidence for greater familial correlation for Vocabulary and Rapid Automatized Naming, and not for Picture Completion, Picture Arrangement, Block Design, and Judgment of Line Orientation is not entirely clear. While those cognitive measures that showed family-specific growth rates were those tests that were verbal in nature, on which performance is often associated with SES (see Sattler, 1992), *post-hoc* analyses indicated that Vocabulary and Rapid Automatized Naming performance was *not* associated with SES. One of the reasons for this finding may have been that our sample was relatively similar in SES. Nonetheless, these findings suggest that the familial association for these certain tests may be intrinsic in nature for this sample; to this end, it has been suggested that verbal abilities are genetically influenced (see Sattler, 1992). Regardless of the reason for this inconsistency between different tests, the fact that we cannot predict which cognitive performances are strongly familial suggests the need to study the specific phenotypic impact of the NF-1 gene on cognition in a familial context. Furthermore, these findings suggest the need to use the same methodology when studying cognitive phenotypes in other genetic disorders.

Despite some of the important implications from this study, there are limitations to the present study. Because of the small sample size, there was possibility insufficient power to detect differences in growth rates between NF-1 and siblings. In addition, while it would have been ideal to estimate variation not only in the level of performance but also the growth of cognitive functioning measures across families, there was insufficient evidence in the data set to estimate growth, so that only level of performance could be estimated. It also should be noted that some of the subjects followed longitudinally were members of the sample upon which earlier reports of the pattern of sparing and impairing of certain cognitive functions in children with NF-1 was based.

Future studies may benefit from examining the longitudinal course of more specific language functions, such as syntax, semantics, and phonology, since reading and language deficits have been shown to be prominent in NF-1 (Cutting et al., 2000b; Mazzocco et al., 1995; North et al., 1997). A more detailed examination of the development of language functions with multiple tests representing one cognitive function (i.e., several tests of vocabulary) could determine if measures examined in this study are actually representative of spared and impaired cognitive *functions* in the NF-1 population (i.e., not just performance on specific *tests*). Thus, future longitudinal studies would not only allow for further clarification of exact deficits (and strengths) in cognitive functions, especially in the language domain, but also greatly aiding in planning early intervention (as discussed above). Another issue for future research is the influence over time of attention deficit hyperactivity disorder (which has recently been confirmed to be much more prevalent in NF-1 than in the general population; Koth et al., 2000) on cognitive functioning in NF-1. In addition, longitudinal cognitive data should be considered in conjunction with neuroimaging data; it may be that different compartments and/or areas of the brain (such as white matter volumes, increased in children/adolescents with NF-1 according to Cutting et al. (2000c) and Said et al. (1996) are differentially associated with performance on spared and impaired tests/functions.

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Megalencephaly in NF1

Predominantly white matter contribution and mitigation by ADHD

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Abstract—*Background:* Megalencephaly is a frequent CNS manifestation in neurofibromatosis type 1 (NF1); however, its tissue composition, modification by attention deficit hyperactivity disorder (ADHD), and relationship with unidentified bright objects (UBO) remain controversial. *Methods:* Eighteen male patients with NF1, seven of whom had ADHD (NF1+ADHD), were compared with 18 age- and sex-matched controls in terms of MRI-, Talairach-based brain, cerebral, lobar, and sublobar gray and white matter volumes. Twelve subjects with NF1 had UBO in the centrencephalic region, whereas six had no UBO or exclusively infratentorial lesions. *Results:* Patients with NF1 without ADHD (NF1-pure) had the largest total cerebral, gray, and white matter volumes with larger parietal/somatosensory white matter volumes than controls, particularly if UBO were present in the basal ganglia. All subjects with NF1 (including NF1+ADHD) had larger total and frontal white matter volumes than controls. Smaller frontal/right prefrontal gray matter volumes were found in NF1+ADHD when compared with NF1-pure patients. *Conclusions:* The increase in frontal and parietal white matter volumes in male patients with NF1, including the preferential centrencephalic distribution, supports the hypothesis that NF1's white matter pathology encompasses but is not limited to visible UBO. Male patients with NF1+ADHD, as compared with NF1-pure patients, showed frontal reductions that are largely consistent with those found in idiopathic ADHD.

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Brain manifestations of neurofibromatosis type 1 (NF1) include tumors,[1] megalencephaly,[1-6] and corpus callosum anomalies.[1,7] On MRI scans there are also often T2-weighted hyperintensities/unidentified bright objects (UBO), which are concentrated in the brainstem, cerebellum, thalamus, and, in particular, in the basal ganglia.[1,2] MRI studies of megalencephaly in NF1 have been contradictory about whether increases in brain and cerebral volume reside in gray or white matter.[4-6] Although megalencephaly has been associated with selective cognitive impairment, its relationship with UBO is unclear.[3,4] Measures of tissue MR signal are beginning to link megalencephaly, UBO, and diffuse cerebral white matter abnormalities in NF1.[6,8-12]

Attention deficit hyperactivity disorder (ADHD) is frequently reported in clinical studies of children with NF1[7,13,14]; one MRI study of NF1 and ADHD found that the anterior body of the corpus callosum was larger in NF1 groups (both with and without ADHD) as compared to controls, but that the posterior midbody was larger only in NF1 without ADHD.[7] Size reductions in rostral and, less often, posterior segments of the corpus callosum have also been reported in boys with idiopathic ADHD.[15,16] These observations suggest that the mechanisms underlying ADHD in NF1 may have a differential effect on brain size, most likely attenuating the increase in cerebral volume described in NF1.[1,3-5]

By means of regional measurement of cerebral gray/white volumes, the current study sought to confirm that enlarged white matter is the main contributor to megalencephaly in children with NF1 and that this cerebral increase might be regionally related to UBO, but that when there is ADHD with NF1 one sees a mitigation of megalencephaly.

Subjects and methods. *Subjects.* We recruited 18 male patients (mean age 11.77 ± 3.83 years) with either familial or sporadic NF1 by NIH criteria[17] and 18 matched male controls (mean age 11.99 ± 4.44). We excluded subjects with intracranial pathology, history of seizures, or uncorrectable hearing or visual impairments. For ADHD diagnosis, subjects had to score positively on two or more of the following measures: the Attention Deficit Hyperactivity Scale from the Diagnostic Interview for Children–Revised–Parent Version, >8/14 items[18,19]; the Dupaul ADHD Rating Scale, ≥2 (on a 4-point Likert scale ranging from 0 to 3) for 6/9 items assessing inattention and/or 6/9 items assessing hyperactivity/impulsivity[20,21]; the Attention Problem Index of the Child Behavior Checklist,[22] with a score of ≥1.5 SDs of the mean (T-score ≥ 65); and the Hyperactivity Index on the Conners Rating Scale–

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Revised–Parent Version,[23] with a score of ≥1.5 SDs of the mean (T-score ≥ 65). Seven subjects with NF1 were diagnosed with ADHD (NF1+ADHD; mean age 10.87 ± 5.03 years). The majority of participants with NF1 were 5 to 14 years and were individually age-matched within 6 months to controls. Control subjects included unaffected siblings as well as subjects without emotional problems or learning disabilities recruited through offices of a local pediatrician. Two controls were African American, one NF1-pure subject was Asian, and the remaining subjects were white. This study was confined to males in order to reduce the number of independent variables in the study, especially when the focus is on neuroimaging volumetrics. Moreover, the mitigation by ADHD of NF1-associated megalencephaly would be different in females with ADHD, whose total brain volume is generally not reduced.[24]

IQ was in the normal range for all subjects (mean full-scale IQ [FSIQ]: controls = 121.41 ± 11.08; NF1: = 98.33 ± 14.62; NF1+ADHD = 90.86 ± 8.17), as determined mainly by the Wechsler Intelligence Scale[25-27] at time of testing. One adult control subject did not have IQ data available, but school records indicated normal aptitude. One subject from the NF1+ADHD group had a FSIQ of 78. One control subject had a previous diagnosis of bipolar disorder and another had a diagnosis of anxiety; both subjects had normal results on MRI. All other control subjects were determined to be free of any psychopathologies. Each participant had parent-signed assent and consent forms that met standards of the Johns Hopkins Medical Institutions' institutional review board.

MRI technique. All scans were performed on a 1.5-T General Electric Signa Scanner (Milwaukee, WI) using the standard GE quadrature head coil. The MR protocol consisted of routine brain MRI scans (sagittal T1 and axial spin-density/T2-weighted) and three-dimensional volumetric radiofrequency spoiled gradient (SPGR) scans with the following parameters: repetition time 35 to 45, echo time 5 to 7, flip angle 45, number of excitations 1, matrix size 256 × 128, and field of view 20 to 24, partitioned into 124 1.5-mm contiguous slices. For most of the NF1 subjects, the routine MRI examination was not part of the initial diagnostic evaluation; however, it served as follow-up scanning for several patients. SPGR scans (5 sagittal, 1 axial, and 30 coronal, approximately equally distributed across groups[3]) were used to obtain volumetric data for each subject.

Spin density/T2-weighted MRI scans showed UBO in 13 NF1 subjects; 11 subjects had one or more UBO in the basal ganglia, and 10/11 also had infratentorial lesions. One subject had UBO in the thalamus only, whereas another had only cerebellar UBO. NF1 subjects with UBO in the basal ganglia or the thalamus were labeled as centrencephalic UBO positive (n = 12) and all other NF1 subjects were labeled as centrencephalic UBO negative (n = 6). Because the volumetric analyses are focused on cerebral changes, we have circumscribed our UBO analyses to this cerebral location (i.e., centrencephalic).

Postacquisition MRI processing, including tissue segmentation into gray, white, and CSF compartments, was carried out as previously described.[28-30] The brain tissue was subdivided into cerebral lobes, subcortical, brainstem, and cerebellar regions according to a revised Talairach stereotaxic grid specific for measurement in pediatric study groups.[31,32] Owing to the reduced validity of subcorti-

cal measurements, only cerebral and lobar volumes were included in the analyses.[31] Based on our hypothesis postulating that fiber bundles originating in frontal and parietal regions are predominantly affected in NF1, particularly in those individuals with basal ganglia and thalamic UBO, the frontal and parietal lobes were subdivided into functional units[33] to further localize the volumetric differences between the groups. The frontal lobe was subdivided into prefrontal, premotor, motor, deep frontal white matter, and anterior cingulate regions as recently reported.[34] Similarly, the parietal lobe was subdivided into the following regions: somatosensory, dorsolateral parietal, inferior parietal, deep parietal white matter, and the posterior cingulate that includes a portion of the corpus callosum.[33] A research assistant who was blind to the diagnosis of each subject carried out all measurements.

Statistical analyses. Univariate (analysis of covariance [ANCOVA]) and multivariate (multivariate analysis of covariance [MANCOVA]) analyses on normalized data were used to compare groups. To control for type 1 error, post hoc tests were conducted only for those multivariate and univariate tests that revealed significance ($p \leq 0.05$). Considering the dynamic nature of volumetric changes throughout childhood,[35-37] for all analyses, age was used as a covariate. Based on our previous study[3] demonstrating larger total cerebral volumes in a large proportion of NF1 subjects, for lobar analyses the latter variable as well as age were used as covariates. As there were no significant correlations with IQ for cerebral volumes, we did not use IQ as a covariate (see Results). Games-Howell post hoc tests, which do not assume equal N or cell normality and can be used for N as small as 6,[38] were used for all post hoc analyses. To reduce type 1 error, the sequence of analyses was decided a priori and was as follows:

1. Total cerebral volume, comparing NF1-pure, NF1+ADHD, and controls
2. Total white and gray cerebral volumes, as in step 1
3. Any significant difference in total cerebral volume derived from step 2 (i.e., gray or white matter) to be further analyzed with regard to lobar divisions
4. Any significant difference in frontal or parietal lobar volume derived from step 3 (i.e., gray or white matter) to be further analyzed with regard to frontal or parietal lobar subdivisions
5. Cerebral, lobar, and sublobar white matter volumes to be compared between those in the NF1 groups (i.e., regardless of ADHD status) with and without UBO in the centrencephalic region

Results. *Preliminary analyses.* Consistent with previous behavioral studies, showing a slight decrease in FSIQ in subjects with NF1,[1,39] particularly those with ADHD,[13] our analyses revealed that the mean FSIQ of the NF1-pure and NF1+ADHD groups were significantly lower than that of the control group. The mean FSIQ of the NF1+ADHD group was lower although not significantly than that of the NF1-pure group. There were no significant correlations with age or FSIQ for total cerebral (gray, white) volumes. There were no significant differences in age; however, given the large age range and the known developmental changes across this age range, especially with regard to white matter,[35-37] we covaried for age.

We also examined the influence of age on total cerebral
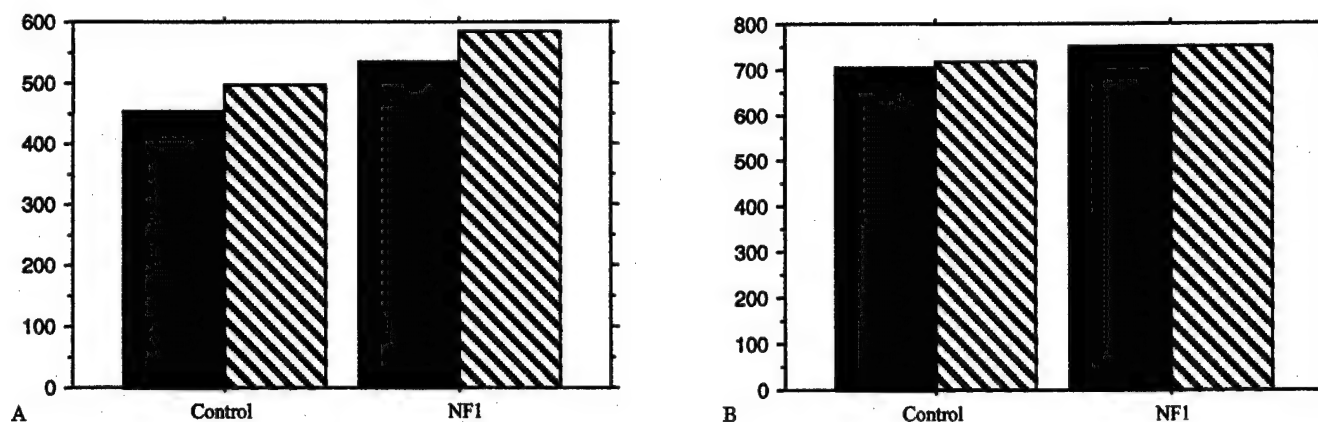
Figure. (A) The bar chart compares total cerebral white matter volumes of the entire neurofibromatosis type 1 (NF1) group, including those with and without attention deficit hyperactivity disorder (ADHD), and their age-matched controls. (B) The bar chart compares total cerebral gray matter volumes of the entire NF1 group, including those with and without ADHD, and their age-matched controls. Black columns = <12 years of age; Shaded columns = >12 years of age.

(gray, white) volumes, by using age as a factor rather than covariate. On the basis of neuroimaging data, demonstrating growth cerebral peaks at about 12 years,[37] we divided the subjects into four groups: NF1 < 12 years of age, NF1 ≥ 12 years of age, controls < 12 years of age, and controls ≥ 12 years of age. The NF1 groups included both NF1-pure and NF1+ADHD subjects. Analyses were conducted between the groups for both total gray and total white cerebral volumes. Results of these analyses revealed that there were significant differences in total white cerebral volumes between the groups split at age 12 (figure). However, the group by age interaction was not significant, indicating that increase in brain volumes occurs in a similar manner between NF1 and control groups (see the figure).

Main analyses. Table 1 summarizes measurements and analyses of total cerebral, total gray cerebral, total white cerebral, and lobar volumes. The ANCOVA for total cerebral volumes revealed that the total cerebral volume of the NF1-pure group was significantly larger (i.e., 13%) than that of NF1+ADHD and that of the control group (i.e., 15.6%) ($F[1, 32] = 8.315$, $p = 0.001$). The total cerebral volume of NF1+ADHD group was 2.6% larger than that of the control group. The MANCOVA for total gray cerebral volumes and total white cerebral volumes was significant; univariate and post hoc analyses revealed that the total gray cerebral volume of the NF1-pure group was larger than that of both the NF1+ADHD and control groups ($F[1, 32] = 6.386$, $p = 0.004$), and the total white cerebral volume of the NF1-pure and NF1+ADHD groups was larger than that of the control group ($F[1, 32] = 10.794$, $p = 0.0003$).

The MANCOVA for white matter lobar analyses was significant; univariate and post hoc tests revealed that the parietal white matter ($F[1, 31] = 5.183$, $p = 0.011$) vol-

Table 1 Cerebral and lobar volumes in NF1 subjects and controls

Regions	NF1—pure (n = 11)	NF1 + ADHD (n = 7)	Controls (n = 18)
Total cerebral volume	1370.94 ± 147.88*	1217.40 ± 52.65	1186.07 ± 101.16
Total cerebral gray matter	789.66 ± 77.44*	691.404 ± 24.43	709.937 ± 65.75
Total cerebral white matter	581.29 ± 82.17†	526.00 ± 41.70†	476.14 ± 47.32
Frontal gray matter‡	278.21 ± 27.76§	242.78 ± 10.48	254.83 ± 24.82
Frontal white matter‡	225.36 ± 36.49†	203.04 ± 17.36†	182.27 ± 19.11
Parietal gray matter‡	191.46 ± 22.63	169.81 ± 12.52	172.05 ± 16.78
Parietal white matter‡	162.21 ± 20.65†	146.18 ± 14.88	132.73 ± 13.57
Temporal gray matter‡	176.19 ± 16.44	150.38 ± 8.54	152.73 ± 16.92
Temporal white matter‡	78.66 ± 12.63	74.80 ± 4.37	65.34 ± 11.70
Occipital gray matter‡	97.12 ± 12.43	82.61 ± 5.88	83.44 ± 8.91
Occipital white matter‡	62.09 ± 10.29	54.51 ± 7.89	52.32 ± 7.251

All values reported as means ± SD. All analyses used age as a covariate.

* $p < 0.01$, Relative to both NF1 + ADHD and controls.
† $p < 0.02$, Relative to controls.
‡ Covaried for total cerebral volume.
§ $p < 0.01$, Relative to NF1 + ADHD.

NF1 = neurofibromatosis 1; ADHD = attention deficit hyperactivity disorder.

Table 2 *Frontal and parietal lobe subdivision volumes in NF1 subjects and controls*

Subdivisions	NF1—pure (n = 11)	NF1 + ADHD (n = 7)	Controls (n = 18)
Frontal subdivisions			
Prefrontal gray matter	139.78 ± 15.77*	117.73 ± 11.90	125.79 ± 13.92
Prefrontal white matter	76.79 ± 18.66	65.41 ± 7.61	57.53 ± 7.66
Premotor gray matter	62.01 ± 7.06	54.92 ± 2.01	56.54 ± 5.73
Premotor white matter	30.96 ± 6.12†	29.86 ± 3.42†	25.20 ± 3.89
Motor gray matter	33.16 ± 4.05	30.05 ± 1.63	29.55 ± 2.98
Motor white matter	24.69 ± 3.76‡	22.85 ± 2.63	20.10 ± 2.87
Frontal deep white matter	63.24 ± 7.73	57.06 ± 4.48	53.82 ± 4.84
Anterior cingulate gray matter	17.03 ± 1.72	15.47 ± 1.91	16.05 ± 2.46
Anterior cingulate white matter	19.66 ± 3.47	18.55 ± 2.13	16.65 ± 2.39
Parietal subdivisions			
Somatosensory gray matter	36.26 ± 4.98	32.44 ± 2.91	31.75 ± 2.98
Somatosensory white matter	20.13 ± 2.85	18.60 ± 1.68	16.12 ± 2.01
Dorsolateral gray matter	52.89 ± 6.90	45.66 ± 3.46	44.52 ± 6.02
Dorsolateral white matter	25.78 ± 4.76	21.92 ± 4.49	19.11 ± 2.92
Inferior parietal gray matter	61.30 ± 7.92	52.68 ± 5.70	53.49 ± 5.49
Inferior parietal white matter	35.68 ± 6.13	31.56 ± 3.75	28.02 ± 3.37
Parietal deep white matter	56.56 ± 6.10	52.17 ± 4.21	49.54 ± 5.14
Posterior cingulate gray matter	14.12 ± 2.52	13.21 ± 2.06	14.48 ± 2.52
Posterior cingulate/corpus callosum white matter	17.49 ± 2.61	16.18 ± 2.03	14.84 ± 1.92

All values reported as means ± SD. All analyses used age and total cerebral volume as covariates.

* $p < 0.03$, Relative to NF + ADHD.
† $p < 0.04$, Relative to controls.
‡ $p < 0.01$, Relative to controls.

NF1 = neurofibromatosis 1; ADHD = attention deficit hyperactivity disorder.

umes of the NF1-pure group were larger than those of the control group, and frontal white matter volumes of both the NF1-pure and NF1+ADHD groups were larger than those of the control group ($F[1, 31] = 5.283$, $p = 0.01$). The MANCOVA for gray matter lobar analyses was significant; univariate and post hoc tests revealed that the frontal gray matter volumes of the NF1+ADHD group were significantly smaller than those of the NF1-pure group ($F[1, 31] = 8.422$, $p = 0.001$).

Table 2 summarizes measurements and analyses of frontal and parietal lobe subdivision volumes. The MANCOVA for white matter frontal subdivision analyses was not significant. In contrast, the MANCOVA for white matter parietal subdivision analyses was significant; univariate and post hoc tests revealed that the posterior dorsolateral white matter volumes of the NF1-pure group were larger than those of the control group ($F[1, 31] = 3.180$, $p = 0.055$), and the somatosensory white matter volumes of both the NF1-pure and the NF1+ADHD groups were larger than those of the control group ($F[1, 31] = 4.823$, $p = 0.015$). The MANCOVA for gray matter frontal subdivision analyses was significant; univariate and post hoc tests revealed that the prefrontal gray matter volumes of the NF1+ADHD group were significantly smaller than those of the NF1-pure group ($F[1, 31] = 4.592$, $p = 0.017$).

Analyses of total white cerebral volumes, frontal lobe,

and parietal lobe white matter in UBO ± groups using the entire NF1 group did not reach significance. However, scatter plots of the data revealed that this was because, as with other findings, the NF1+ADHD group resembled the controls. In order to preliminarily explore if presence of UBO impacted parietal and frontal white matter volumes, a Robust rank order test was conducted within the NF1-pure group alone, of whom eight had UBO in the basal ganglia and three did not. This revealed significant differences in total white cerebral volumes ($p < 0.05$), parietal white matter ($p < 0.05$), motor white matter ($p < 0.01$), frontal deep white matter ($p < 0.05$), and somatosensory white matter ($p < 0.05$), such that the white matter of those with UBO was more markedly enlarged.

Considering data demonstrating abnormal patterns of brain symmetry in idiopathic ADHD[15,16] and other conditions associated with ADHD (i.e., Tourette's syndrome),[39,40] we also analyzed asymmetry indices for the four lobes (based on data in table 3). The MANCOVA was not significant, indicating similar asymmetry relationships between groups. An additional MANCOVA was also conducted to determine if the lobes for which there were significant differences between groups in gray and white matter were specific to the right or left side. The result of this MANCOVA was significant; univariate and post hoc tests revealed differences for right parietal white matter ($F[1,31] = 7.828$, $p =$

Table 3 *Lobar volumes for left and right hemispheres in NF1 subjects and controls*

Regions	NF1—pure (n = 11)	NF1 + ADHD (n = 7)	Controls (n = 18)
Frontal gray matter			
Left	134.73 ± 15.71*	117.56 ± 5.65	122.63 ± 13.13
Right	143.48 ± 13.33*	125.22 ± 5.91	132.19 ± 12.25
Frontal white matter			
Left	111.59 ± 18.67	101.401 ± 11.70	91.295 ± 9.28
Right	113.77 ± 18.02†	101.636 ± 7.98†	90.968 ± 11.02
Parietal gray matter			
Left	93.40 ± 11.41	83.02 ± 6.65	85.15 ± 8.49
Right	98.06 ± 11.83	86.80 ± 6.63	86.90 ± 8.61
Parietal white matter			
Left	80.44 ± 12.33	72.99 ± 7.55	66.41 ± 8.12
Right	81.76 ± 8.60	73.18 ± 7.45	66.32 ± 5.90
Temporal gray matter			
Left	87.78 ± 9.06	74.15 ± 4.59	74.92 ± 9.17
Right	88.40 ± 8.16	76.23 ± 4.05	77.81 ± 8.00
Temporal white matter			
Left	39.64 ± 6.39	37.57 ± 1.76	32.96 ± 6.30
Right	39.01 ± 6.79	37.23 ± 3.26	32.37 ± 5.80
Occipital gray matter			
Left	47.85 ± 6.43	41.11 ± 3.50	41.83 ± 4.43
Right	49.27 ± 6.44	41.50 ± 3.08	41.61 ± 4.89
Occipital white matter			
Left	32.37 ± 5.75	26.94 ± 4.01	25.96 ± 4.53
Right	29.72 ± 4.96	27.57 ± 4.68	26.36 ± 3.51

Values reported as means ± SD. All analyses used age and total cerebral volume as covariates.

* $p < 0.01$, Relative to NF + ADHD.

† $p < 0.02$, Relative to controls.

NF1 = neurofibromatosis 1; ADHD = attention deficit hyperactivity disorder.

0.001), right frontal white matter ($F[1,31] = 4.488$, $p = 0.019$), and both left and right frontal gray matter ($F[1,31] = 4.617$, $p = 0.017$ and $F[1,31] = 8.994$, $p = 0.0008$), with the NF1-pure group showing significantly larger right parietal white matter volumes than the controls. Both NF1 groups (NF1-pure and NF1+ADHD) displayed larger right frontal white matter volumes than the control group, with the NF1-pure group exhibiting increases in right frontal gray matter as compared to the NF1+ADHD group. Table 3 displays measurements and analyses of lobar volumes by hemisphere.

We extended the abovementioned analyses to the subdivisions of the frontal and parietal lobes to further localize the lobar hemispheric differences to specific regions; these significant findings are shown in table 4. The MANCOVA for the frontal white matter subdivisions was significant; univariate and post hoc tests revealed that the right motor white matter ($F[1,31] = 3.812$, $p = 0.003$) and the right premotor white matter ($F[1,31] = 4.554$, $p = 0.018$) were larger for the entire NF1 group (both NF1-pure and NF1+ADHD) as compared to controls. The MANCOVA for the parietal white matter subdivisions was significant; univariate and post hoc tests revealed that the right somatosensory white

matter was larger for both NF1-pure and NF1+ADHD patients as compared to controls ($F[1,31] = 5.421$, $p = 0.009$), and the right dorsolateral parietal white matter and the inferior parietal white matter were larger for the NF1-pure group as compared to controls ($F[1,31] = 4.023$, $p = 0.028$ and $F[1,31] = 6.501$, $p = 0.004$, respectively). The MANCOVA for the frontal gray matter subdivisions was significant; univariate and post hoc tests revealed that right prefrontal gray matter was smaller for NF1+ADHD as compared to NF1-pure patients ($F[1,31] = 4.307$, $p = 0.022$).

Discussion. In this MRI volumetric study, we confirmed that males with NF1 (NF1-pure and NF1+ADHD) are indeed megalencephalic (larger total cerebral volume). This cerebral enlargement appeared to be attributable to the cerebral white matter, in particular to the frontal white matter, and of greater magnitude in NF1-pure patients. The latter group was also characterized by parietal/somatosensory white matter enlargement, particularly if UBO were present in the basal ganglia. Comorbidity with ADHD not only led to a mitigation of parietal

Table 4 *Hemispheric findings in the frontal and parietal lobe subdivision volumes in NF1 subjects and controls*

Subdivisions	NF1—pure (n = 11)	NF1 + ADHD (n = 7)	Controls (n = 18)
Frontal subdivisions			
Right prefrontal gray matter	72.31 ± 7.57	61.53 ± 6.04	65.78 ± 7.09
Right premotor white matter	15.52 ± 3.09*	15.24 ± 2.01*	12.30 ± 2.35
Right motor white matter	12.73 ± 1.90†	11.65 ± 1.46	10.20 ± 1.59
Parietal subdivisions			
Right somatosensory white matter	10.07 ± 1.36†	9.31 ± 0.68†	8.02 ± 1.07
Right dorsolateral parietal white matter	12.93 ± 2.43*	11.22 ± 2.39	9.53 ± 1.48
Right inferior parietal white matter	18.35 ± 2.42†	15.68 ± 1.96	13.91 ± 1.58

All values reported as means ± SD. All analyses used age and total cerebral volume as covariates.

* $p < 0.03$, Relative to controls.
† $p < 0.01$, Relative to controls.

NF1 = neurofibromatosis 1; ADHD = attention deficit hyperactivity disorder.

increase in NF1 subjects, but NF1+ADHD patients had smaller frontal/right prefrontal gray matter volumes than NF1-pure subjects. Frontal and parietal volume changes involved predominantly the right hemisphere. When considering overall findings relevant to NF1, the white matter volume appears to be the predominant contributor to cerebral enlargement; however, gray matter volume also contributes to megalencephaly and is decreased only in those individuals with comorbid ADHD.

The data presented here add to the growing literature[3-6] demonstrating that in NF1 macrocephaly[1] is the result of increased total brain and cerebral volumes. In agreement with previous publications,[5,6] our results support our original postulate of a selective increase in white matter cerebral volume. Discrepancy between our findings and a prior report[4] might be attributed to the basis of study design. Inclusion of NF1 males without tumors and, in particular, classification of their ADHD status allowed the identification of ADHD as a factor that lessens cerebral, frontal gray, and parietal white matter enlargements. These data are in agreement with the demonstration of segmental callosal enlargement only in NF1 subjects without ADHD,[7] and with literature on males with idiopathic ADHD suggesting selective callosal, frontal, and parietal reductions.[15,16,41] A predominant right hemispheric change in NF1 is also in line with brain morphometric work on related conditions such as ADHD[15,16,40] and Tourette's syndrome.[39,40]

Our study showed that cerebral white matter enlargement in NF1 involves primarily the frontal and parietal lobes. This pattern does not appear to contradict the diffuse nature of cerebral white matter abnormality in NF1[2,6,8,12]; rather, as hypothesized, cerebral white matter abnormality in NF1 may be related to the development of UBO, because our preliminary analyses showed that greater white matter enlargements are associated with centrencephalic UBO. Recent MR studies have provided links

between megalencephaly, diffuse cerebral white matter abnormalities, and UBO in NF; more sensitive MRI sequences than those employed in this investigation show widespread distribution of UBO,[42-44] reductions in T1 signals that characterize some UBO are more marked in NF1 patients with white matter enlargement,[6] and decreases in *N*-acetylaspartate/choline ratios are not confined to areas of UBO.[8] AQ: 9 Genetic studies have also suggested a link between the NF1 gene and primary involvement of myelin in this condition.[45-47] MR studies integrating different modalities as well as behavioral data, particularly applying a longitudinal design, would be instrumental in further elucidating the nature and functional repercussions of white matter abnormalities in NF1.

Acknowledgment

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Longitudinal Evolution of Unidentified Bright Objects

in Children with Neurofibromatosis-1

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Running Title: UBO evolution in Neurofibromatosis-1

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(ABSTRACT)

Neurofibromatosis type-1 (NF-1) is the most common autosomal dominant disorder affecting the central nervous system. Magnetic resonance imaging (MRI) has revealed distinctive T2-weighted hyperintense foci (termed UBOs), which appear to represent spongiform changes in the white matter. Cross-sectional and longitudinal analyses suggest that UBOs disappear over time; however, none of these studies have examined comprehensively these foci. We conducted a quantitative MRI longitudinal study of number of affected regions, number of UBOs per region, and UBO volume per region, in a sample of 12 children with NF-1. We applied semi-automatic morphometric methods and comprehensive statistical approaches, within a detailed anatomical parcellation framework. Our data demonstrate that, despite a similar UBO regional distribution (e.g., prevalent globus pallidus/internal capsule location), UBO evolution was more complex than previously reported. The total number of UBO-occupied locations evolved in a non-linear manner, with a decrease between approximately ages 7-12 years, followed by a progressive increase during adolescence. This pattern was also found for UBO number and/or volume for all regions, with the exception of the cerebellar hemispheres. This distinction may reflect differences in white matter structure between affected long tract fiber bundles and that of cerebral and cerebellar myelinated fibers. The findings are also discussed in the context of previous MR and behavioral studies. We conclude that studies like the present one, in association with other MR modalities, are necessary to characterize more completely the nature and evolution of UBOs and their role in the cognitive phenotype of NF-1.

Key Words: Neurofibromatosis-1, UBO, MRI, evolution

INTRODUCTION

Neurofibromatosis type-1 (NF-1) is the most common autosomal dominant disorder affecting the central nervous system [Riccardi, 1999]. Since the earliest magnetic resonance imaging (MRI) studies of patients with NF-1 [Brown et al., 1987; Hurst et al., 1988; Aoki et al., 1989; Goldstein et al., 1989], investigators have noted the presence of T2-weighted hyperintense foci, also termed unidentified bright objects (UBOs), most often found in the deep gray structures, the brainstem, and the cerebellum. The histologic nature of the abnormalities remains obscure, although the limited available pathologic material suggests that the signal abnormalities reflect spongiform changes in the white matter [DiPaolo et al., 1995], compatible predominantly with intramyelinic edema. At least two groups have evaluated the evolution of these foci over time [Itoh et al., 1994; DiMario and Ramsby, 1998], and have found that, in general, the UBO burden tends to decrease over time, but that the patterns of change over time vary by brain region. In one of the previous studies, in which patients were examined serially, seven patients were studied at more than two time points [DiMario and Ramsby, 1998]. Those results were reported in a framework of an anatomic parcellation in which the locations of all the foci were assigned to cerebral hemisphere, brainstem (diencephalic UBOs were included in this compartment) or cerebellum; using this parcellation scheme, the UBOs were found to occur most often in the cerebellum and in the globus pallidus. The authors alluded to the fact that their data showed an increase in the number and size of brainstem foci over the course of several years, while the UBO burden in the cerebral hemispheres and the cerebellum decreased. In the other study, Itoh et al. [1994] serially studied 13

patients as part of a larger investigation into the evolution of the NF-1-related abnormalities. In that study, the anatomic distinctions were somewhat finer, with five compartments (basal ganglia, brainstem, dentate nuclei, cerebellar white matter, cerebral white matter) to which UBOs could be assigned. They found the foci occur most frequently in the basal ganglia and in the brainstem and that, with time, the overall tendency was for the UBOs to decrease in size. The methods used to characterize the time-dependent behavior of the UBOs were not well described in either study. Also, while the study by Itoh et al. [1994] characterized the UBO evolution in terms of their volume, the DiMario and Ramsby's report focused on region specific UBO number and maximal cross sectional area, without explicit calculation of their volumes. Furthermore, postmortem [DiPaolo et al., 1995] and MRI [Said et al., 1996; Steen et al., 2001] studies have emphasized the importance of white matter involvement in NF-1, including the fact that UBOs appear to be distributed along major white matter tracts [Yamanouchi et al., 1995]. The latter data, in conjunction with our own observations, suggest that the distribution and evolution of UBOs should be analyzed in a scheme of anatomical parcellation that accounts for white matter fiber bundles that originate or terminate at, or pass through, the regions being evaluated.

The nature and functional significance of UBOs in NF-1 remain controversial; detailed characterizations of the evolution of these MRI abnormalities will contribute to the clarification of these issues. The goal of this study was to analyze **anatomically** these NF-1 abnormalities **from a longitudinal perspective**, in a pediatric sample with predominantly more than two observations and

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within a higher-resolution MRI anatomic framework, in order to characterize more accurately both their spatial distribution and the time course of their evolution. Since some aspects of the behavioral deficits exhibited by NF-1 patients, specifically the lowering of IQ relative to unaffected siblings, appear to correlate with the number of regions involved by UBOs [Denckla et al., 1996], the present investigation examined all three variables, i.e., number of UBOs, size of UBOs, and number of regions affected by these UBOs. Determining similarities and differences in the time courses of region-specific UBO number and volume would be helpful in not only characterizing the neuroanatomical bases of the behavioral abnormalities exhibited by children with NF-1, but also would shed some light into the nature of the underlying pathogenetic process. **Characterization of the regional distribution of UBOs, as well as of the relationship between number and size of these abnormalities, over time would help to elucidate whether the UBO process is focal or diffuse and whether dynamic changes are region-specific.** We postulate that this information, in the context of a detailed anatomical framework, will contribute to both more informative UBO-deficit correlations and better clinical prognostication in NF-1.

MATERIAL AND METHODS

Population and Procedures

We obtained 34 examinations on twelve patients (eleven males, one female) with NF-1, ranging in age from about 7.1 to about 19.5 years, with an average (+/- s.d.) age of 13.0 +/- 3.3 years and average full scale IQ of 105.0 +/- 12.3. Five subjects were evaluated at two time points, four subjects at three time points, and three at four time points. The mean number of scans/subjects was 2.8, and the mean **overall** time interval between scans was 2.0 years **(time 1-time 2: mean, 1.98; range, 0.1-3.6; time 2-time 3: mean, 2.39; range, 1.0-3.0; time 3-time 4: mean, 2.20; range, 1.4-2.9). Table I provides basic information about the longitudinal assessment of the subjects under study.**

Insert Table I about here

The subjects were recruited as part of a larger research-center-based investigation of genetic and learning disabilities, **entitled** "Neurodevelopmental Pathways to Learning Disabilities", at the Kennedy Krieger Institute in Baltimore, Maryland. **Subject inclusion in the present study was based on completeness of behavioral and imaging evaluations rather than on UBO status during the first MRI.** Children affected with either familial or sporadic NF-1 were diagnosed based on the National Institutes of

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Health consensus criteria [NIH. National Institutes of Health Consensus Development Conference, 1988]. **Five subjects were diagnosed with sporadic NF-1 and seven were diagnosed with familial NF-1.** Subjects were excluded if they had any intracranial pathology such as optic gliomas and/or other brain tumors, history of seizures, or any significant uncorrectable hearing or visual impairments. Cognitive ability was assessed with the Wechsler Intelligence Scale for Children [Wechsler 1974, 1991] at the time of the first MRI scan. Nine children were assessed with the Wechsler Intelligence Scale for Children- revised version (WISC-R) and three were assessed with the Wechsler Intelligence Scale for Children- III (WISC-III).

MRI Acquisition

All scans were performed on a 1.5 T General Electric Signa Scanner (Milwaukee, Wisconsin) using the standard GE quadrature head coil. Three axial series were obtained parallel to the anterior-posterior (AC-PC) intercommissural line: a T1-weighted sequence (TR/TE=500-600/20), as well as the proton density (PD) and T2-weighted sequences (TR/TE = 3000/30,100 ms) that were used for UBO evaluation. All series were gathered as 5 mm thick interleaved images with a field of view of between 22-24 cm. The imaging data were then post-processed to reduce systematic inhomogeneities within and between scan sections as previously described [Denckla et al., 1996].

MRI Post-Acquisition Analysis

The UBOs, defined operationally as regions of confluent hyperintensity (signal intensity higher than that of cortical gray matter) on the PD and the T2-weighted images without associated mass effect, were initially identified by visual inspection by two of the authors independently. For each imaging section in which foci were detected, UBOs were initially manually delineated, followed by a seed-based automatic segmentation algorithm that provided the final outline of the UBO [Itoh et al., 1994; Denckla et al., 1996]. Because of the complex three-dimensional configuration of UBOs, we used the operational designation of independent focus for every abnormality observed on every single MRI slice. **Table I depicts the number of UBOs per subject in each evaluation.** This UBO definition was used for both number and volume measurements. The foci were assigned to one of eighteen pre-defined anatomic regions (**Table II**), reflecting both the patterns of UBO distribution in previous reports [Aoki et al., 1989; Itoh et al., 1994; Yamanouchi et al., 1995; Steen et al., 2001], as well as in our data. If a UBO crossed regional boundaries, it was counted or measured in each of the locations. These regions assignments were reviewed, and any uncertainties regarding anatomic localization were resolved by adjudication between two of the authors, one of whom is a Neuroradiologist (MAK) and the other a Neuropathologist (WEK).

Insert Table II about here

Since the number of UBOs per hemisphere was approximately the same for all regions (see Results), for all analyses the left and right hemispheres were combined. Number of UBOs represented the count of independent UBOs per each one of the eighteen regions under evaluation **(Table II)**. Preliminary examination of these raw data demonstrated that UBOs were rather infrequent in some of the original locations. In addition, distinction between UBOs in the globus pallidus and internal capsule was rather difficult in many instances. Therefore, original regions were combined into clusters representing locations with similar function and/or containing different portions of the same fiber bundle system **(Table III)**. **Consequently, all the analyses** of number of locations, number of UBOs, and UBO volume **reported here** were conducted on the following **clusters**: (1) striatal region: caudate, putamen, claustrum, external capsule; (2) globus pallidus, internal capsule; (3) diencephalic region: thalamus, hypothalamus, subthalamus; (4) medial cerebellar region: cerebellar vermis, deep cerebellar nuclei, middle cerebellar peduncle; (5) ventral midbrain (cerebral peduncle), ventral pons; (6) dorsal midbrain, pontine tegmentum, medulla; **and (7) lateral cerebellar hemispheres (mainly white matter), which were analyzed as a single entity because of the relatively large number of UBOs in this location**. From this scheme, number of locations was defined as the total number of any of the seven clusters/region occupied by UBO(s) per MRI examination. Volumetric assessments were done by reconstruction of a volume of interest (VOI) as described in earlier studies [Itoh et al., 1994; Denckla et al., 1996]. Each VOI (in cc) represented the addition of all UBO volumes within a given anatomical region (the seven clusters/regions mentioned above); in other words, it did not correspond to the volume of a single focus but rather to the combined volume of one or

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more UBOs per location. Figure 1 depicts UBOs in the **frequently occurring** globus pallidus/internal capsule location.

Insert Table III about here

Insert Figure 1 about here

Statistical Plan and Analysis

All analyses were preformed using STATA 6.0.

Number of locations and UBOs. We postulate that number of locations would reflect the severity and dynamics (e.g., progressive or static) of the pathophysiologic process. Complementing the latter, number of UBOs per specific location would represent the regional propensity for UBO formation and, to some extent, the "activity" of the process. Both continuous variables were analyzed by a Poisson regression analysis, in which the outcome is the incidence rate of the event. In this case, the incidence is the number of locations per subject, or the number of UBOs, expressed as a function of age and age[2]. The incidence rate for an individual j can be expressed in general terms as:

$$r_j = \exp(\beta_0 + \beta_1 age_j + \beta_2 age_j{}^2)$$

where the regression beta coefficients are estimated from the data. Because we have multiple measurements taken on the individual patients, the method of general estimating equations (GEE) was used with the STATA procedure command *xtgee* and the Poisson distribution specified. The ratio of the incidence rates allows us to calculate incidence rate ratios (IRR) between two individuals. For instance, we may want to know whether a one-year increase in age increases or decreases the expected number of locations or UBOs. In the case of the cerebellar hemispheres, the incidence rate was found to be linear in age only with a decrease in expected UBOs over time (e.g., β_2 in the above equation is statistically equal to zero), giving a incidence rate ratio for each one-year increase as:

$$IRR = \exp(-0.4247) = 0.654,$$

where -0.4247 is our estimated value for β_1 in the above equation.

This tells us that the expected rate of UBOs for a given patient is 0.654 times that of another patient that is one year **older**, regardless of the ages being compared.

In the cases where the quadratic term in age is significant, then the actual ages being compared effects the estimated IRR. For example, the non-linear incidence rate of UBOs in the globus pallidus/internal capsule region (see Fig. 4) produced an IRR for an 11 year old versus a 10 year of 0.861, and an IRR for a 17 year old versus a 16 year old as 1.154. Thus, a one year increase in age from 10 to 11 results in a lower expected number of UBOs, while a year increase in age from 16 to 17 results in an increase in the expected number of UBOs according to the regression model.

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<u>UBO volume</u>:   We hypothesize that region-wise volume of UBO would reflect the magnitude **or severity** of the spongiform change.   Preliminary scatter plots of UBO volumes on age fitted with a smoothing spline, suggesting that UBO volumes are not linear with age, but instead are high at younger ages, decrease into the early teenage years, and then increase again in the later teen years.   Therefore, UBO volumes for a given location were regressed on linear and quadratic terms in age (e.g., independent variables of age and age^2) as suggested by the data pattern in the scatter plots.   The regression model can be written in general terms as:

$$total\,volume = \beta_0 + \beta_1 age + \beta_2 age^2$$

where the regression beta coefficients are estimated from the data.   As for the counts described above, the GEE was used with the STATA procedure command *xtgee* that allowed to estimate the regression coefficients and corresponding standard errors of these coefficients while taking into account possible correlations in UBO volumes within the individual.

RESULTS

Regional distribution of UBOs

The 34 MRI examinations in our 12 subjects demonstrated a total of 336 UBOs, as defined in the Methods section, in the regions under analyses. The number of UBOs in other locations was substantially lower. The most frequent location was the globus pallidus/internal capsule with a total of 210 UBOs (left, 94; right 116). **The second most common locations were the diencephalic and medial cerebellar regions, with 47 and 40 UBOs respectively (left, 20; right, 27; and left, 17; right, 23). The cerebellar hemispheric white matter showed 33 UBOs (left, 19; right, 14), while the total number of UBOs in the striatal cluster was 28 (left, 15; right, 13). Finally, the combined count of UBOs for the two brainstem regional groups was 77 (left, 37; right, 40).**

Number of locations affected by UBOs

Analysis of the number of clusters with at least one UBO, as a function of age, showed a non-linear trend. The number of affected regions was initially high (approximately 5), decreased during the pre-teen years (between ages 5 and about 13 years to an approximate mean of 2.5), and increased again in the late teens to a level comparable to early childhood. Figure 2 illustrates this evolution.

_____

Insert Figure 2 about here

_____

Numbers of UBOs

As mentioned in the preceding Methods section, we found two distinct patterns of UBO appearance/regression into which focus number segregate. In the cerebellar vermis, deep cerebellar nuclei, middle cerebellar peduncle cluster, as well as in the cerebellar hemispheric white matter, the number of UBOs drops sharply from between the ages of 5 and 10 years, and remains small thereafter. Data demonstrating this pattern is shown in Figure 3. In all other locations, UBO number demonstrates the same non-linear pattern of evolution shown by the number of involved regions (see preceding section and Fig. 2). There was an initial pre-teen decrease followed by a similar increase between approximately ages 13 and 19 years. This prevalent pattern is exemplified by the location with largest numbers of UBOs, the globus pallidus/internal capsule, which is depicted in Figure 4.

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Insert Figure 3 about here

_____

Insert Figure 4 about here

Volume of UBOs

In the case of total UBO volume per region, most locations showed a definite non-linear trend in age, where UBO volume was initially high, decreased between ages 7 and 12-14 years, and increased again thereafter. As with the number of UBOs, an exception to this rule was the region of the cerebellar hemispheres. The regression analyses demonstrated that UBO volume was linear with age, with a statistically significant decrease as the patients get older. In the striatal region, neither the age nor the age^2 coefficients met strictly the criteria for statistical significance, but the covariates closely approached significance with corresponding p-values of 0.051 and 0.052 for age and age^2, respectively. Figures 5 and 6 illustrate the linear and non-linear patterns, by depicting UBOs volume for the cerebellar hemispheres and the globus pallidus/internal capsule, respectively.

Insert Figure 5 about here

Insert Figure 6 about here

Relationship between Number and Volume of UBOs

Considering that our operational definitions of number and volume of UBOs were based on abnormalities per slice and per region, respectively, and that more than one region could be represented on each slice and several slices may contribute to a single region, we directly examined the relationship between the UBO variables. Regression analyses of the entire sample for two of the most common regions, the globus pallidus/internal capsule and the cerebellar hemispheres, showed a direct relationship between UBO number and volume. Although the exact relationship or slope for each patient was different, there was a clear trend towards an increase in volume with increasing number of UBOs (coefficient and p values of 0.48 and <0.01 and 0.43 and <0.01 for globus pallidus/internal capsule and cerebellar hemispheres, respectively).

Graphs depicting the statistical functions for number and volume of UBOs over time, in Figures 3-6, show that although there was a high correspondence between UBO number and volume for the entire group, the agreement was not complete. The latter was particularly the case for the cerebellar hemispheres (number, Fig. 3a; volume, Fig. 5a).

Effect of atypical subjects

We also examined the influence upon UBO number and volume of the two subjects (#238 and #1276, Table I) who did not display abnormalities throughout the longitudinal study, as well as of the only female patient (#186, Table I). The exclusion of these individuals did not affect the findings reported for the globus pallidus/internal capsule and the cerebellar hemisphere clusters. These regions demonstrated the same characteristic non-linear and linear decreasing patterns of UBO number and volume, respectively, which were mentioned above.

DISCUSSION

Our data demonstrate the same overall regional distribution of the characteristic T2W hyperintense foci reported by previous studies in children with NF-1. The globus pallidus/internal capsule was the most common location, with substantially fewer UBOs in the diencephalic, medial cerebellar, hemispheric cerebellar, and striatal regions. Only the number of UBOs in the brainstem was relatively smaller than earlier reports [DiMario and Ramsby, 1998]. Despite this general agreement, we found a different and complex pattern of evolution of UBOs. In terms of the number of affected regions, UBOs are initially found in most of the examined locations (i.e., approximately 5 out 7 regional clusters). This is followed by a decrease to a minimum (approximately 2.5 clusters) during the pre-teen years, and a progressive increase to earlier childhood levels after age 12-14 years. This non-linear evolution is also observed for both the number of UBOs per region and the total volume occupied by UBOs in a given region, in most locations. Interestingly, not only the pattern but also the specific ages mentioned above are similar for most of the parameters. A distinct exception to this pattern of longitudinal evolution was evident in the cerebellar hemispheres, which showed a statistically significant decrease in both UBO number and volume as the patients got older. Additionally, the number of UBOs in the medial cerebellar structures (i.e., cerebellar vermis, deep cerebellar nuclei, middle cerebellar peduncle), displayed a linear reduction over time, while UBO volume in the striatal region (caudate, putamen, claustrum, external capsule) showed a trend approaching significance towards non-linear evolution ($p = 0.052$ for age^2).

The data presented here provide, to our knowledge, the first evidence of a complex pattern of evolution for UBOs in children with NF-1. These UBOs have been recognized as a distinctive feature of NF-1 since the late 1980s [Aoki et al., 1989; Goldstein et al., 1989; Mirowitz et al., 1989; Yamanouchi et al., 1995; North, 2000]. Cross-sectional analyses, and more recently longitudinal evaluations, indicate that UBOs tend to regress during adolescence [Itoh et al., 1994, DiMario and Ramsby, 1998; North, 2000]. The first major serial study of UBOs included 13 subjects with two observations each, and assessed volume of UBOs in five locations: cerebral white matter, basal ganglia, brainstem, dentate nucleus, and cerebellar white matter [Itoh et al., 1994]. In 7/13 subjects, UBOs disappeared or decreased; two individuals showed no change, and 3 subjects had new or larger foci. Interestingly, in this study, one NF-1 subject showed UBO volume increase in the basal ganglia and reduction in the brainstem and cerebral white matter [Itoh et al., 1994]. These data suggest that while the predominant pattern is one of decrease, there are NF-1 patients who may experience appearance or enlargement of UBOs. A second serial investigation incorporated a true longitudinal component, with 7/15 patients with more than two MRI scans over time [DiMario and Ramsby, 1998]. These authors quantified both numbers and size (i.e., maximal diameter) of UBOs in three locations: hemispheres (e.g., globus pallidus), cerebellum (including deep cerebellar nuclei), and brainstem (including diencephalic structures). They found that while number and size of hemispheric and cerebellar UBOs decreased with age, those in the brainstem appear to increase [DiMario and Ramsby, 1998]. The discrepancy between these results, in particular those referring to the hemispheric UBOs, and our data could be explained by

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several factors. **In addition to a higher proportion of females in the sample (8/30 vs. 1/12 in our study) and to a wider age range (1-53 years vs. 7-14 years in the present study),** the proportion of the sample with more than two observations is slightly larger in our analyses (58% vs. 47%). **Therefore, we** postulate that our more comprehensive statistical analyses may have better recorded the changes over time in UBO parameters. An example of the strength of comprehensive longitudinal statistical approaches is the morphometric work **on normal cerebral development** conducted by Giedd and colleagues [1999]. These authors demonstrated cerebral gray matter volumetric increases throughout childhood (to a peak in adolescence), which have not been disclosed previously by cross-sectional analyses [Giedd et al., 1996; Reiss et al., 1996]. In support of our findings of non-linear evolution of UBOs, other investigators have reported that T1-weighted signal hyperintensities associated with the typical UBOs [Mirowitz et al., 1989] tend to appear later in childhood and do not regress [Terada et al., 1996].

In addition to our more comprehensive statistical analytical strategy, the present study included a detailed anatomical parcellation. The latter separated related brain regions, which are distinct in terms of function or white matter structure (i.e., the most affected tissue component by UBOs). Despite this effort, we found, as did DiMario and Ramsby [1998], only two different regional patterns of evolution. One is characterized by decrease of foci over time, which parallels the results reported by the two studies mentioned in preceding paragraphs, was the least common and was evident predominantly within the cerebellar hemispheres. Itoh et al. [1994] and DiMario and Ramsby [1998] also reported reductions of UBOs in the cerebellum; however, the latter

authors did not differentiate medial structures such as the deep nuclei from the cerebellar hemispheres. In our data, medial cerebellar regions showed a mixed behavior; while the number of foci declined linearly with age, the volume of UBOs displayed a non-linear decrease-increase pattern. Another finding from our study, with relative agreement with the previous longitudinal study [DiMario and Ramsby, 1998], was the evolution of brainstem and diencephalic (mainly thalamic) UBOs. The latter authors grouped both regions, which demonstrated an increase in UBOs over time. We also found an increase in UBOs; however, this phenomenon was only seen during early adolescence.

The results presented here suggest that UBOs are a dynamic type of abnormality, which not only develops during early childhood but also can appear during adolescence. The fact that of all locations only the cerebellar white matter shows a different evolution, with the previously reported decrease over time, may be a reflection of the unique nature of the UBO phenomenon. Whereas there is ample evidence that UBOs represent the "visible" manifestation of a more widespread process, as we demonstrated by MR spectroscopy (MRS) [Wang et al., 2000] and Steen and colleagues [2001] by T1 relaxometry, affecting predominantly the white matter [Itoh et al., 1994; DiPaolo et al., 1995; Yamanouchi et al., 1995; Said et al., 1996; Steen et al., 2001], the vast regions of cerebral hemispheric white matter are minimally involved [Itoh et al., 1994; Yamanouchi et al., 1995]. The exception seems to be the cerebellar white matter, which is frequently affected by the UBO process [Itoh et al., 1994]. We postulate that the distinctive distribution of UBOs, predominantly along major fiber bundles and centroencephalic regions [i.e., globus pallidus, thalamus] is linked to the compact packing of myelinated

fibers in these locations [Carpenter, 1976]. Our recent diffusion tensor imaging (DTI) study of brainstem fiber bundles revealed that each major tract has a unique set of MR parameters, including differences in MR T2 relaxation times [Stieltjes et al., 2001]. These MR properties may be in part responsible for the MRI features of UBOs. It appears that the less compact and more heterogeneous architecture of cerebral and cerebellar white matter make them less susceptible to develop long-term UBOs. The fact that all three parameters evolved in a decrease-increase pattern, for most regions, suggests that initially developed multi-focal UBOs tend to regress in number and size and that the post-puberty increase simply represent an inverse process to the pre-teen involution. Whether the process corresponds to a "re-activation" of former foci or the development of new UBOs is unclear, at this point, and deserves further examination.

The functional significance of UBOs is still a controversial issue. While there is evidence associating the presence of UBOs with many measured cognitive impairments [reviewed by North, 2000], the differential impact of UBO number and size is unclear. Denckla and colleagues [1996], using a model that predicts "lowering" of IQ relative to unaffected siblings, found that the number of UBO-occupied locations and not the proportion of tissue involved by UBOs or age was a significant predictive factor. In this regard our results, showing an increase in the number of affected locations during early adolescence, could help to re-evaluate the approach to UBO-deficit relationships in pediatric NF-1. In terms of association between UBO location and behavioral impairment, our findings do not provide additional insight. For instance, the reported

correlation between thalamic UBOs and cognitive deficit [Moore et al., 1996] does not appear to be a reflection of a unique evolution of diencephalic UBOs.

In summary, this study on the longitudinal profile of UBOs in children with NF-1 indicates that these foci do regress during late childhood but reappear in early adolescence. Although this information is of great importance from the diagnostic viewpoint, taking into account the number of examined subjects **and gender distribution**, our investigation should be considered preliminary in nature. As in other quantitative neuroimaging studies [Giedd et al., 1999], we have taken advantage of the statistical power of longitudinal data, in which each sequential observation increases the strength of the data. Nonetheless, extension of the approach presented here to larger datasets will contribute to a more complete view of UBO evolution. Finally, integration of longitudinal UBO morphometry data with other quantitative neuroimaging methods, such as DTI, MRS, and T1 and T2 relaxometry, will ultimately lead to a better understanding of UBO pathophysiology and to specific therapeutic interventions.

ACKNOWLEDGMENTS

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UBO evolution in Neurofibromatosis-1

TABLE I. Total number of UBOs in all regions for each subject at every time point

Subject	Age/Time 1	UBOs/Time 1	Age/Time 2	UBOs/Time 2	Age/Time 3	UBOs/Time 3	Age/Time 4	UBOs/Time 4
95	10.1	5	13.3	5	15.9	5	--	--
97	7.1	19	7.3	51	9.7	30	12.6	5
184	11.3	14	14.9	9	16.5	11	--	--
186[a]	11.0	8	13.6	5	16.7	12	--	--
238	10.2	0	13.1	0	14.1	0	16.4	0
316	13.5	10	15.1	5	18.1	25	19.5	21
347	14.2	4	17.0	8	--	--	--	--
644	7.4	10	8.8	8	--	--	--	--
965	11.8	1	14.2	5	17.2	1	--	--
1094[b]	13.1	3	14.9	5	--	--	--	--
1276	11.7	0	12.9	0	--	--	--	--
1651	8.6	29	8.7	22	--	--	--	--

[a] Female subject
[b] Atypical UBO distribution (no UBOs in globus pallidus/internal capsule or cerebellar hemispheres)

TABLE II. Anatomic Subdivisons For UBO Localization

Region 1:   cerebral hemispheres (white matter)

Region 2:   corpus callosum

Region 3:   caudate

Region 4:   putamen

Region 5:   globus pallidus

Region 6:   claustrum/external capsule

Region 7:   internal capsule

Region 8:   thalamus

Region 9:   hypothalamus and subthalamus

Region 10:   (lateral) cerebellar hemispheres (white matter)

Region 11:   cerebellar vermis

Region 12:   deep cerebellar nuclei

Region 13:   dorsal midbrain

Region 14:   ventral midbrain (cerebral peduncles)

Region 15:   dorsal pons

Region 16:   ventral pons

Region 17:   middle cerebellar peduncle

Region 18:   medulla

TABLE III. Clusters of Anatomic Subdivisons For UBO Localization

Cluster 1:   striatal region: caudate, putamen, claustrum, external capsule

Cluster 2:   globus pallidus, internal capsule

Cluster 3:   diencephalic region: thalamus, hypothalamus, subthalamus

Cluster 4:   medial cerebellar region: cerebellar vermis, deep cerebellar nuclei, middle

                cerebellar peduncle

Cluster 5:   ventral midbrain (cerebral peduncle), ventral pons

Cluster 6:   dorsal midbrain, pontine tegmentum, medulla

Cluster 7:   (lateral) cerebellar hemispheres (white matter)*

_____

(*): This cluster consists of only one region
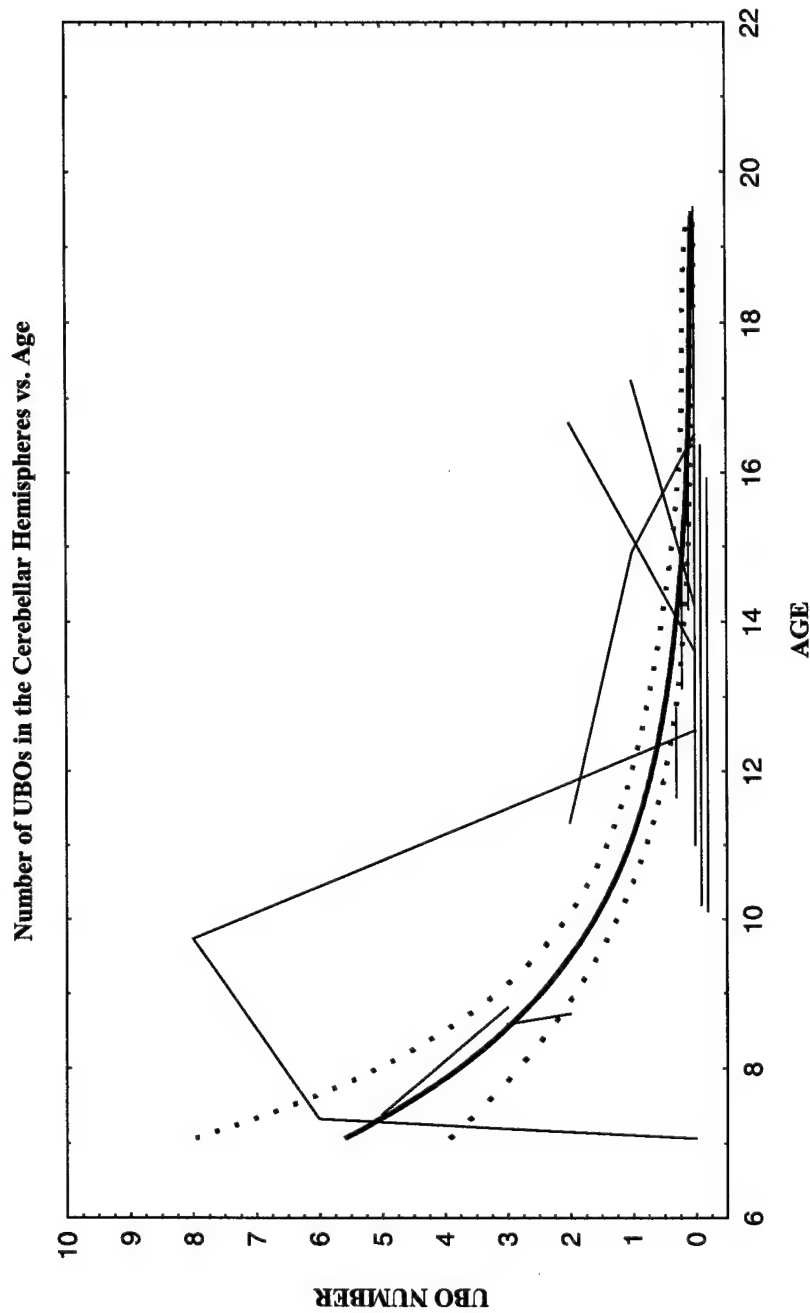
Kraut et al.

b.

a.
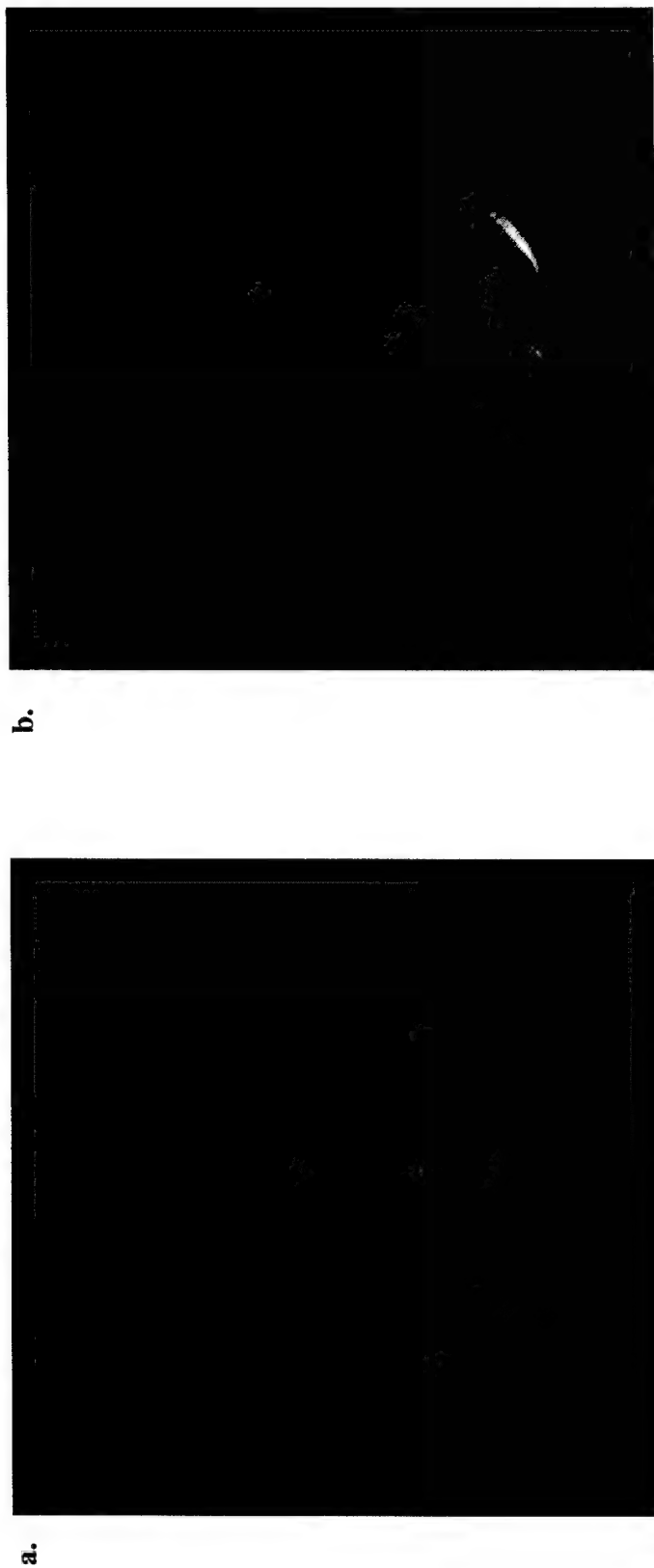
Figure 1. Proton density MRI scan showing UBOs on the right and left globus pallidus/internal capsule region. B. Same MRI sequence showing UBOs on both medial (left) and lateral (right) cerebellar regions.

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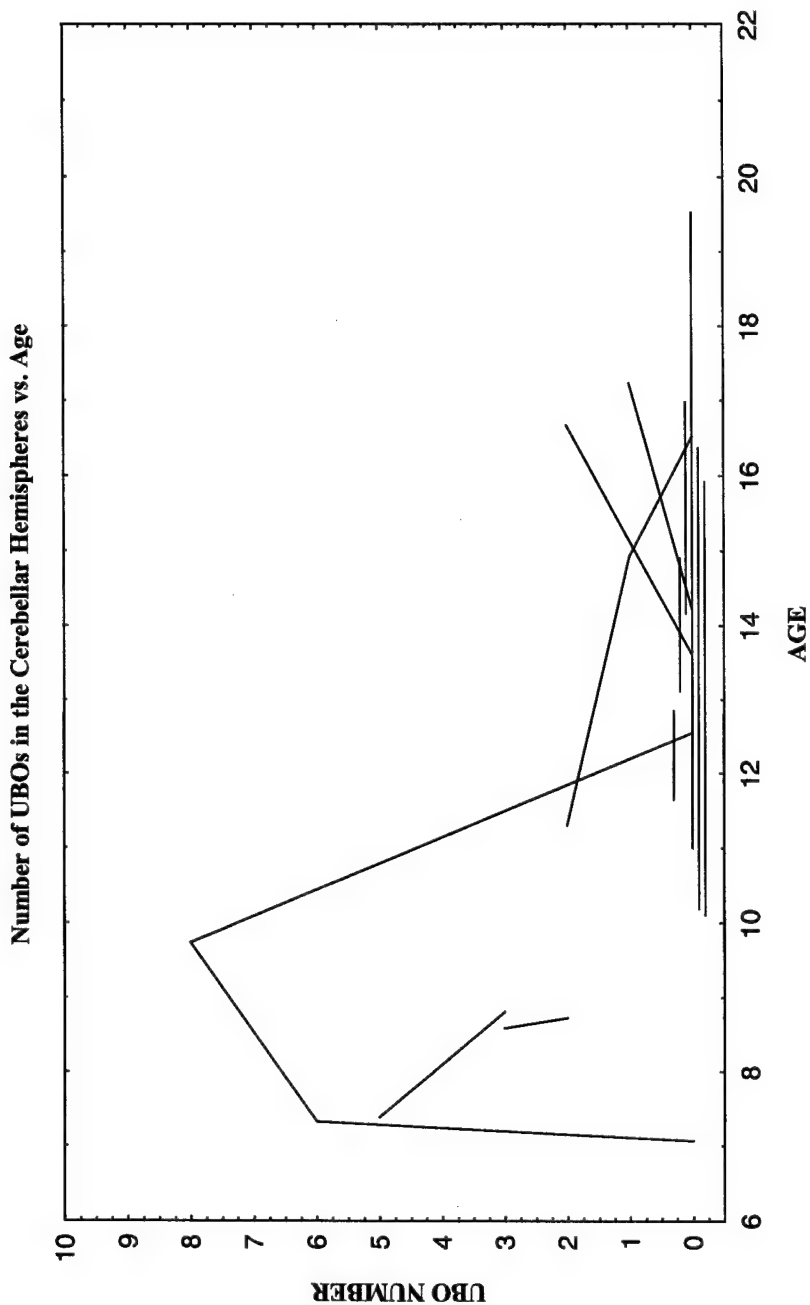
Locations affected by UBOs vs. Age
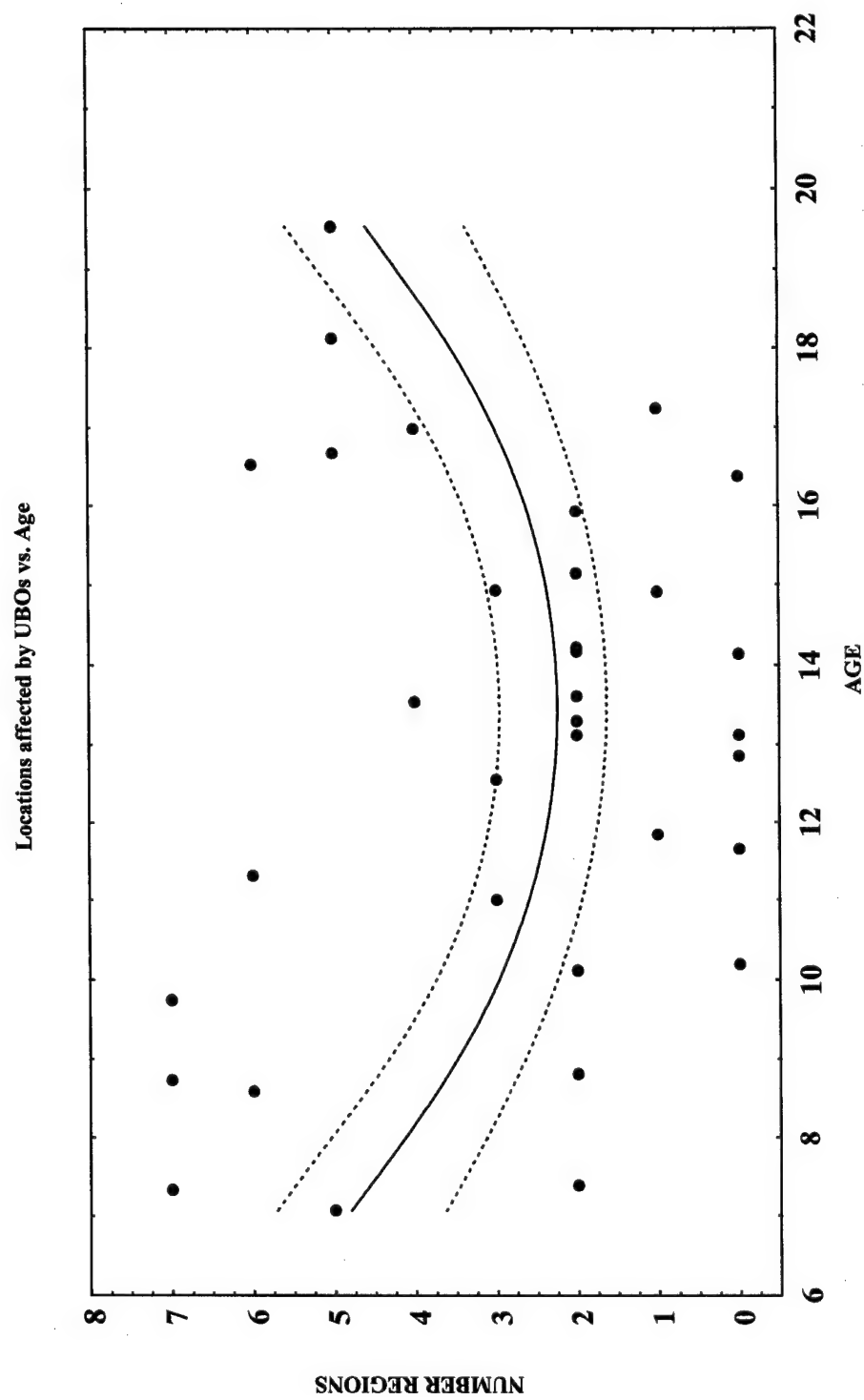
Figure 2. Number of UBO-occupied regions in function of age. Note the non-linear evolution of UBO distribution, with relatively high initial levels that decrease until 12-14 years of age, followed by a progressive increase during adolescence.

UBO evolution in Neurofibromatosis-1

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a.

Number of UBOs in the Cerebellar Hemispheres vs. Age

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UBO evolution in Neurofibromatosis-1

b.

Number of UBOs in the Cerebellar Hemispheres vs. Age

Figure 3. Number of UBOs in the cerebellar hemispheres (white matter) in function of age. (a) Individual trajectories of number of UBOs for each subject and regression curve for the entire group (bold), including confidence intervals (dotted lines). (b) Same

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UBO evolution in Neurofibromatosis-1

individual trajectories of number of UBOs per subject. Note the relatively linear evolution with steady decrease after high levels in early childhood.

UBO evolution in Neurofibromatosis-1

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Number of UBOs in the GP/IC region vs. Age

a.

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UBO evolution in Neurofibromatosis-1
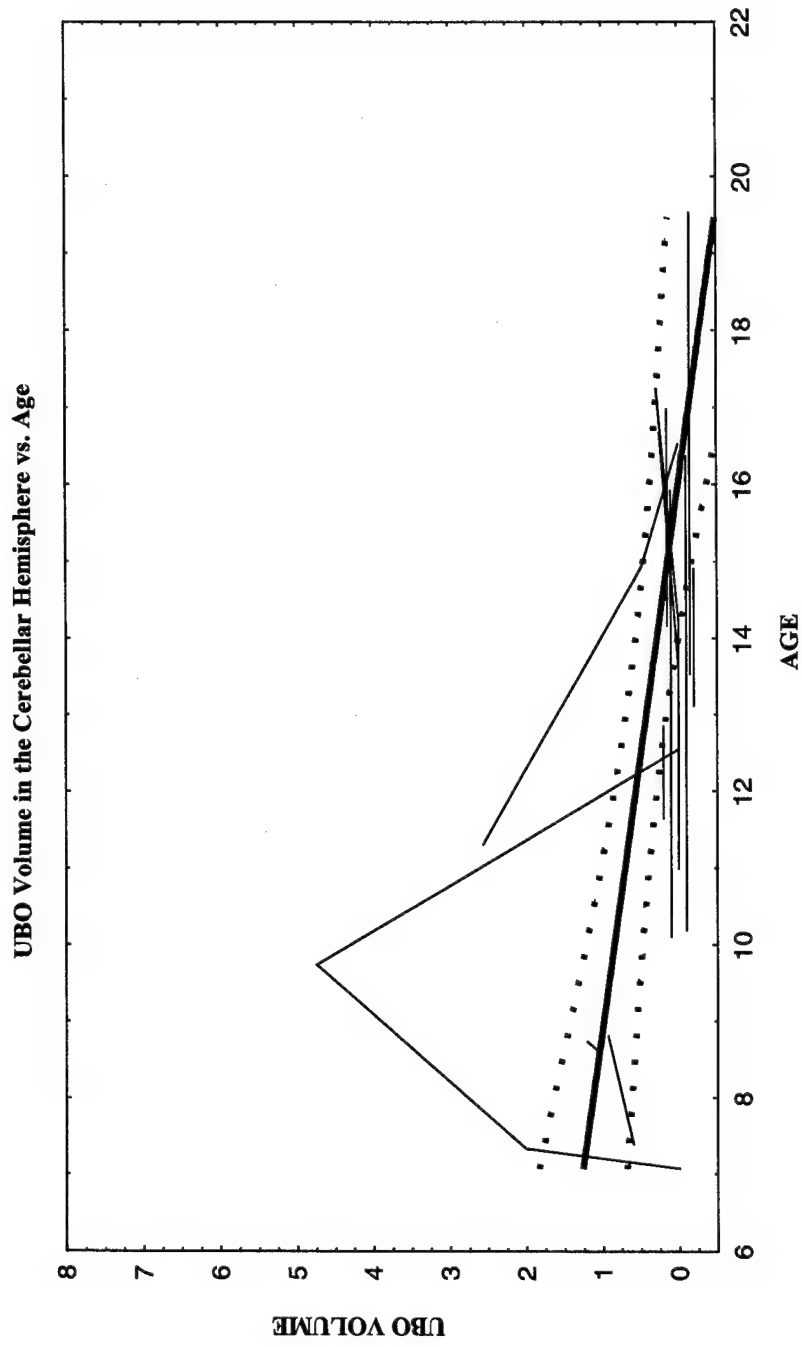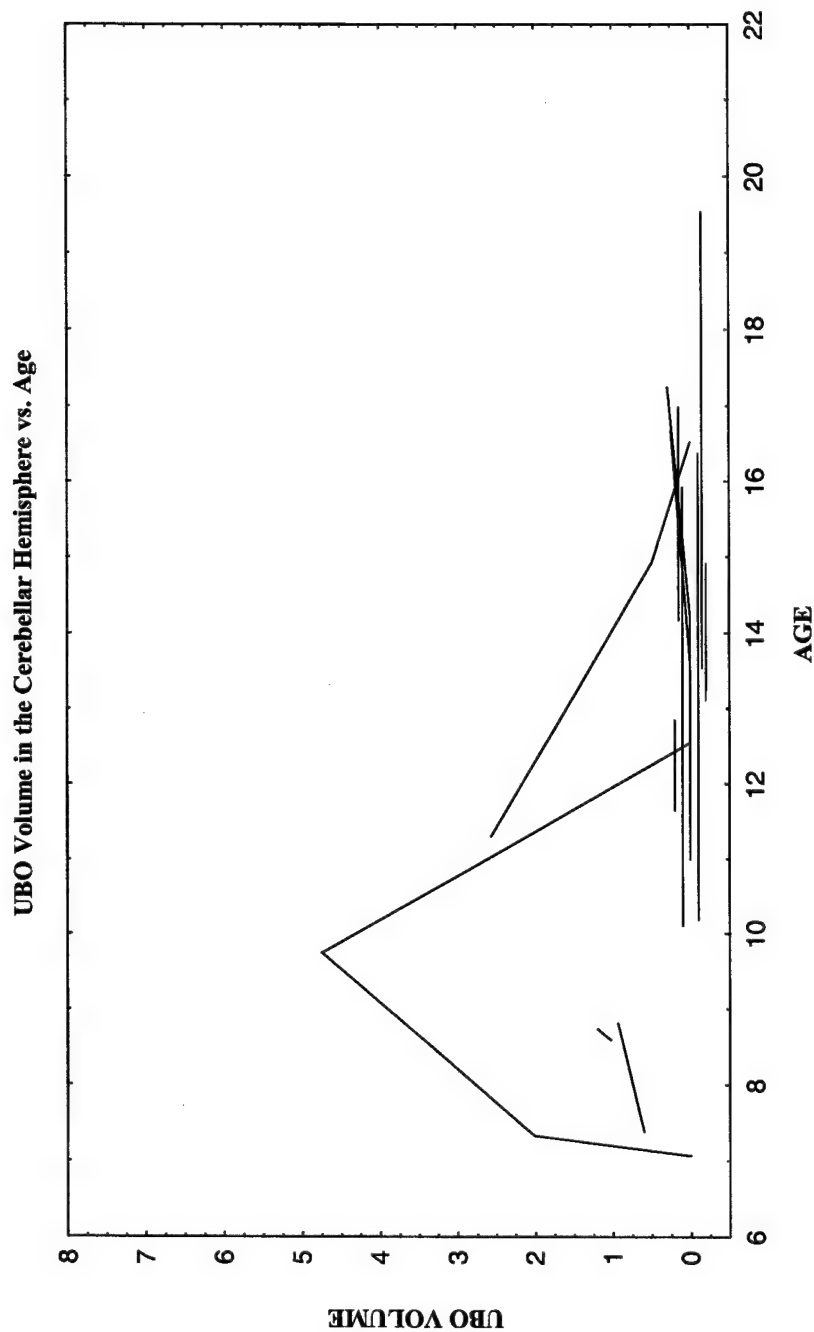
b.

Number of UBOs in the GP/IC region vs. Age
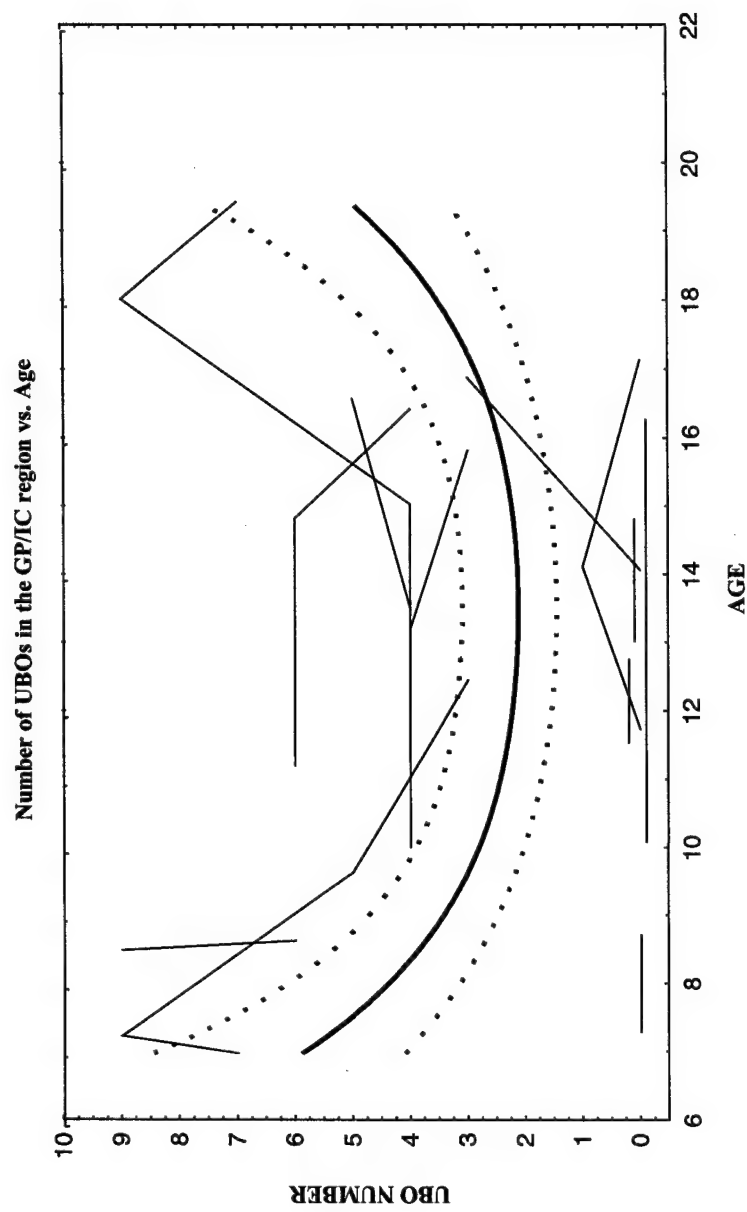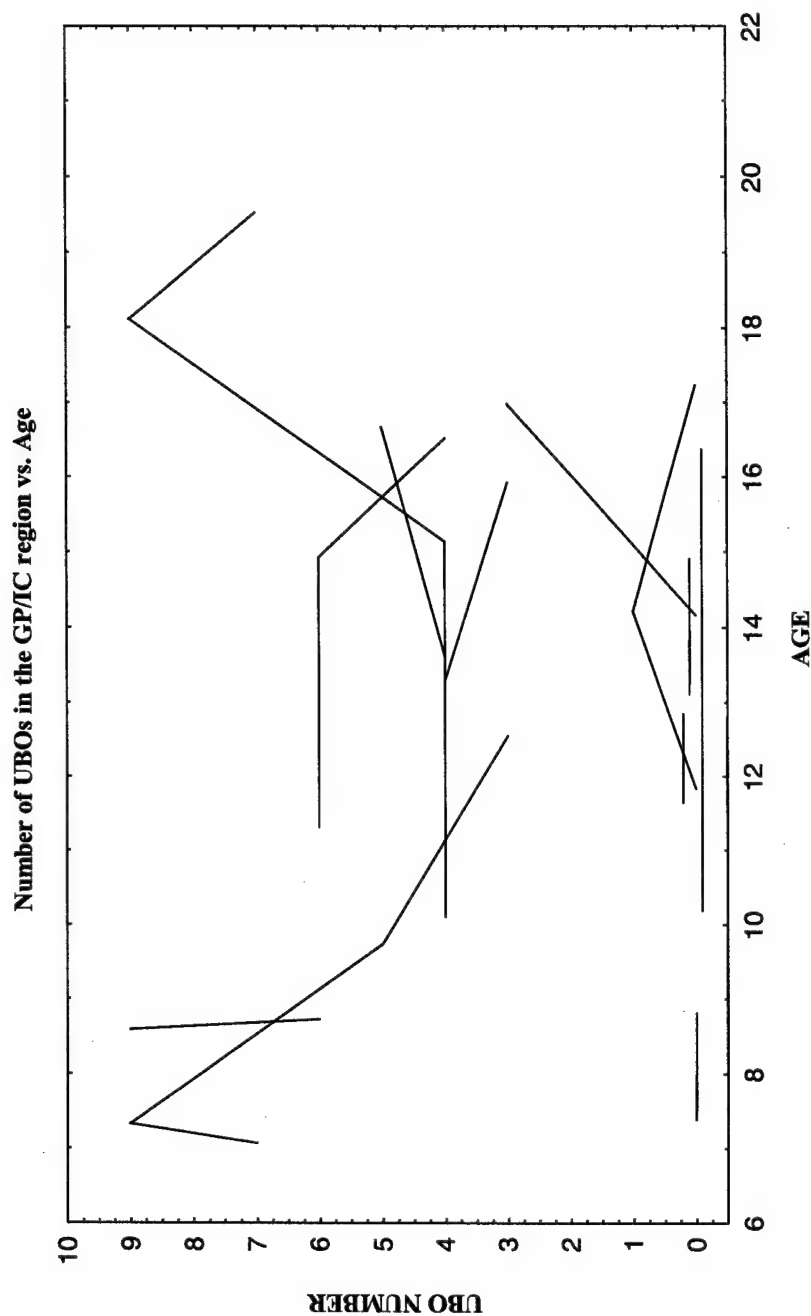
Figure 4. Number of UBOs in the globus pallidus/internal capsule (GP/IC) region in function of age. (a) Individual trajectories of number of UBOs for each subject and regression curve for the entire group (bold), including confidence intervals (dotted lines). (b) Same individual trajectories of number of UBOs per subject. Note the non-linear evolution of UBOs with early decrease and late post-puberty increase, which resembles longitudinal pattern of UBO locations (see Fig. 2).

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a.

**UBO Volume in the Cerebellar Hemisphere vs. Age**

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b.

Figure 5. Volume of tissue (in cc) occupied by UBOs in the cerebellar hemispheres (white matter) in function of age. (a) Individual trajectories of volumes of UBOs for each subject and regression curve for the entire group (bold), including confidence intervals (dotted lines). (b) Same individual trajectories of volumes of UBOs per subject. Note the linear decrease since early childhood.
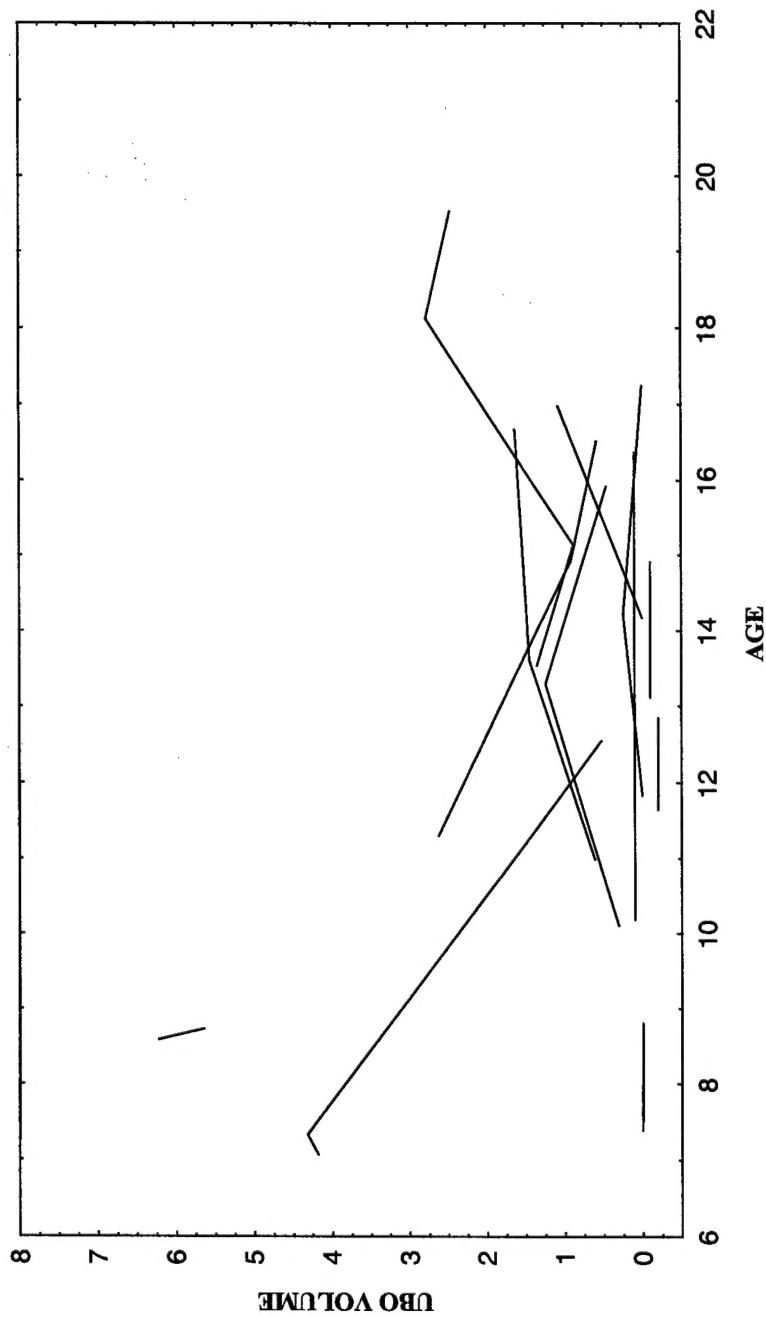
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a.
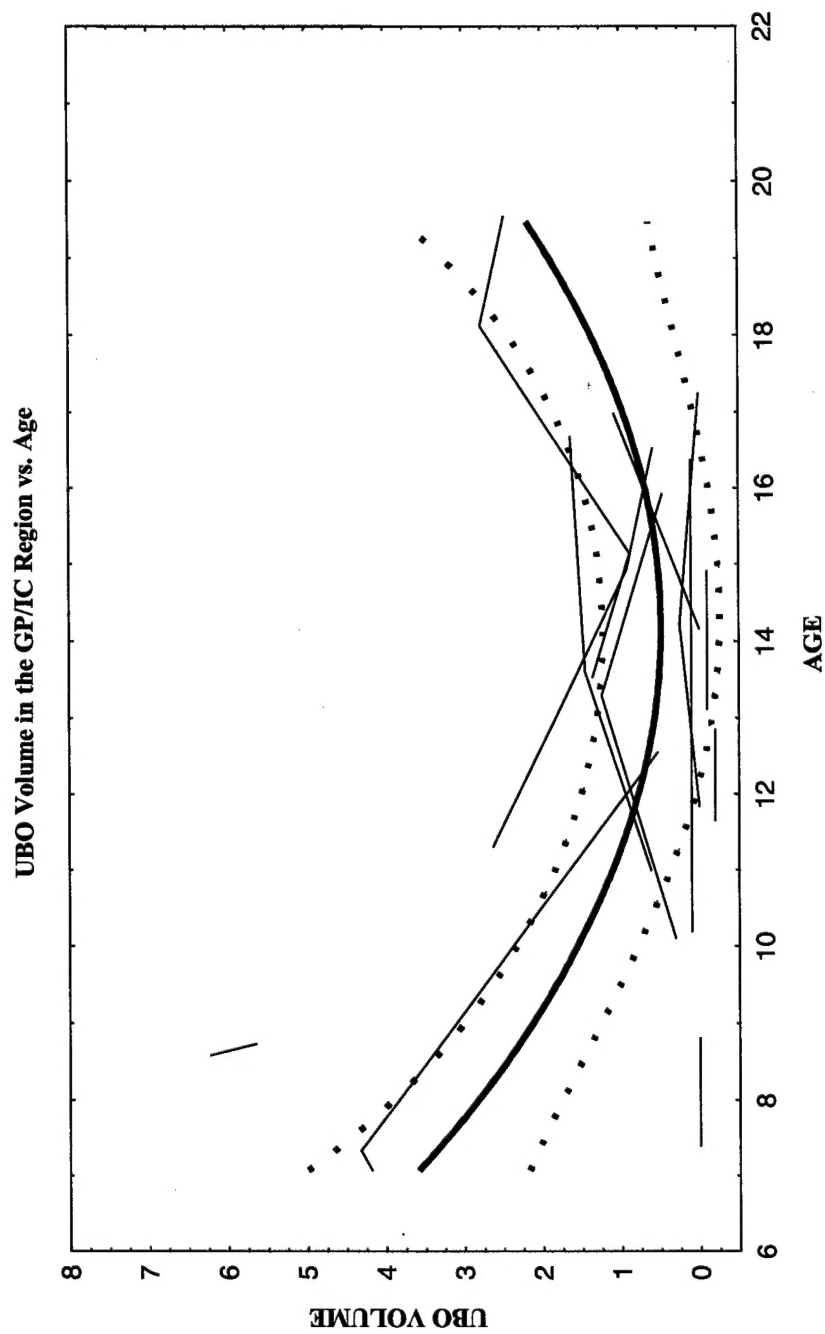
UBO Volume in the GP/IC Region vs. Age

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UBO evolution in Neurofibromatosis-1

b.

Figure 6. Volume of tissue (in cc) occupied by UBOs in the globus pallidus/internal capsule (GP/IC) region in function of age. (a) Individual trajectories of volumes of UBOs for each subject and regression curve for the entire group (bold), including confidence intervals (dotted lines). (b) Same individual trajectories of volumes of UBOs per subject. Note the non-linear evolution of relatively

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UBO evolution in Neurofibromatosis-1

large UBOs volumes in early childhood, which decrease to a minimum between 12-14 years of age, followed by a post-pubertal increase. A similar pattern of evolution can be seen in Figs. 2 and 4.